Insulin-induced cytokine production in macrophages causes insulin resistance in hepatocytes

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Manowsky J, Camargo RG, Kipp AP, Henkel J, Püschel GP. Insulin-induced cytokine production in macrophages causes insulin resistance in hepatocytes. Am J Physiol Endocrinol Metab 310: E938–E946, 2016. First published April 19, 2016; doi:10.1152/ajpendo.00427.2015.—Overweight and obesity are associated with hyperinsulinemia, insulin resistance, and a low-grade inflammation. Although hyperinsulinemia is generally thought to result from an attempt of the β-cell to compensate for insulin resistance, there is evidence that hyperinsulinemia itself may contribute to the development of insulin resistance and possibly the low-grade inflammation. To test this hypothesis, U937 macrophages were exposed to insulin. In these cells, insulin induced expression of the proinflammatory cytokines IL-1β, IL-8, CCL2, and OSM. The insulin-elicted induction of IL-1β was independent of the presence of endotoxin and most likely mediated by an insulin-dependent activation of NF-κB. Supernatants of the insulin-treated U937 macrophages rendered primary cultures of rat hepatocytes insulin resistant; they attenuated the insulin-dependent induction of gluco kinase by 50%. The cytokines contained in the supernatants of insulin-treated U937 macrophages activated ERK1/2 and IKKβ, resulting in an inhibitory serine phosphorylation of the insulin receptor substrate. In addition, STAT3 was activated and SOCS3 induced, further contributing to the interruption of the insulin receptor signal chain in hepatocytes. These results indicate that hyperinsulinemia per se might contribute to the low-grade inflammation prevailing in overweight and obese patients and thereby promote the development of insulin resistance particularly in the liver, because the insulin concentration in the portal circulation is much higher than in all other tissues.

metabolic syndrome; type 2 diabetes; inflammation; macrophage; insulin; cytokines

Despite tremendous efforts to raise public awareness about potential health hazards, the prevalence of overweight and obesity continues to increase (22). Concurrently, the incidence of impaired glucose tolerance, insulin resistance, and the often ensuing type 2 diabetes is rising at a stunning rate. Several molecular mechanisms have been proposed to explain the development of insulin resistance in obese patients. Thus, accrual of lipids with second-messenger properties in the course of ectopic triglyceride accumulation, namely diacylglycerol, can activate protein kinases, e.g., protein kinase C. This may result in the interruption of the intracellular insulin receptor signal chain by an inhibitory serine phosphorylation of the first relay protein in this chain, the insulin receptor sub-

* J. Manowsky and R. G. Camargo contributed equally to this work.
MATERIALS AND METHODS

Materials. Medium M199, antibiotics, newborn calf serum, FCS, and polymyxin B were purchased from Biochrom (Berlin, Germany). Medium RPMI 1640 was obtained from Gibco (Eggenstein, Germany) and Vector pGL3-basic from Promega (Mannheim, Germany). Insulin, dexamethasone, phorbol 12-myristate 13-acetate (PMA), GSK1838705, and lipopolysaccharide (LPS) were purchased from Sigma-Aldrich (Deisenhofen, Germany). Acetone and oligo(dT)12–18 were obtained from Roth (Karlsruhe, Germany). RevertAid M-MuLV reverse transcriptase and Maxima SYBP Green quantitative PCR (qPCR) Master Mix were purchased from Thermofisher Scientific (Darmstadt, Germany). Antibodies against phospho-Akt (Ser473), Akt, phospho-p44/42 MAPK (Thr202/Tyr204), p44/42 MAPK, phospho-Ser329-IRS-1, phospho-Ser636/639-insulin receptor substrate-1 (IRS-1), IRS-1, phospho-IKKα/β (Ser176/177), IKKB, phospho-IkBα (Ser32/36), IkBα, phospho-STAT3 (Tyr705), STAT3, and phospho-p44/42 MAPK (Thr202/Tyr204) were dissolved in 55 g/ml of lysis buffer per culture plate [20 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% (vol/vol) Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM NaF, protease inhibitors, and 1 mM sodium orthovanadate] and homogenized by sonication, and insoluble material was removed by centrifugation (10,000 g, 15 min, 4°C). The protein content was determined, and SDS-PAGE as well as Western blot analysis were performed as described previously (9) with phospho-specific and pan antibodies (see Materials). For detection of IL-1β in supernatants of U937 macrophages, medium was mixed with acetone 1:5, stored overnight at −20°C, and centrifuged (4,696 g, 5 min, 4°C). The pellet was dissolved in 55 µl of loading buffer [400 mM Tris-HCl, pH 6.8, 10% (wt/vol) SDS, 25% (vol/vol) glycerol, and 0.125% (wt/vol) bromophenol blue] and used for Western blot analysis.

Real time RT-PCR analysis. RNA was isolated from rat hepatocytes and macrophages using thepeqGold Total RNA Kit (Peqlab, Erlangen, Germany). cDNA was synthesized from 600 to 1,500 ng of purified RNA with 500 ng oligo(dT)12–18 using 200 U of RevertAid M-MuLV reverse transcriptase according to the manufacturer’s instructions. Hot start real-time PCR for the quantification of each transcript was carried out in triplicates in a reaction mixture of 2 × SybrGreen qPCR Master Mix, 250 nM forward and reverse oligo-
cleotides, and 0.3 μl cDNA in a total volume of 10 μl. qPCR was performed with an initial enzyme activation step at 95°C for 10 min, followed by 50 cycles of denaturation at 95°C for 20 s, annealing at 57–60°C for 30 s, and extension at 72°C for 20 s with a subsequent melt curve analysis in a CFX96 Thermal Cycler (Bio-Rad, Munich, Germany). The expression level was calculated as an n-fold induction of the gene of interest (int) in treated vs. control cells with β-actin (ref) as a reference gene. The calculation is based on the differences in the threshold cycles between control (c) and treated (treat) groups according to the formula: fold induction = 2(c-treat)/2(c-treatref).

Statistical methods. All data are expressed as means ± SE. Differences between the groups were analyzed by Student’s unpaired t-test and, where appropriate, with two-way ANOVA with Tukey’s test for multiple comparison and considered statistically significant when P < 0.05.

RESULTS

Insulin-dependent induction of cytokine production in U937 macrophages. Human U937 cells were differentiated into macrophages by an established standard protocol with PMA. The macrophage differentiation markers CD11b and CD163 were induced significantly by this treatment (Table 1). U937 cells were also incubated with 100 nM insulin. Insulin neither induced a differentiation into macrophages on its own nor impacted on the PMA-dependent differentiation. U937 cells that were differentiated into macrophages in absence of insulin were then treated with 100 nM insulin for 24 h. Insulin treatment significantly induced the expression of IL-1β twoto 2.5-fold both on the mRNA in cell lysates and on the protein level in cell culture supernatants (Fig. 1, A and B). Similar results were obtained with another human monocyte/macrophage cell line, THP-1 cells, or if lower insulin concentrations were added repeatedly (at 0, 12, 16, and 20 h) to the U937 cells (control: 1.00 ± 0.30; 4 × 10 nM insulin: 1.30 ± 0.35, P = 0.003; 4 × 1 nM: 1.14 ± 0.28, P = 0.063). To exclude that the IL-1β induction in macrophage-differentiated U937 was either a consequence of an LPS contamination of the insulin used to stimulate the cells or a consequence of the synergism of insulin with low levels of LPS in the media, the experiment was repeated in presence of polymyxin B to bind and inactivate LPS. Cells were stimulated with LPS as positive control. Although the roughly 3.5-fold induction of the IL-1β mRNA by LPS was completely abolished in presence of polymyxin B, the significant 2.5-fold induction by insulin was not affected by polymyxin B (Fig. 1C), excluding the possibility that the observed insulin effect was independent of the presence of LPS. To further corroborate the hypothesis that the induction of IL-1β was dependent on insulin receptor signaling, experiments with the insulin receptor inhibitor GSK1838705 were performed. In support of the hypothesis, GSK1838705 completely abolished the insulin-dependent induction of the IL-1β mRNA in U937 macrophages (Fig. 1D).

The IL-1β promoter contains an AP1 and an NF-κB-binding site. Both elements have been implicated in the regulation of IL-1β expression (12, 25). Therefore, it was tested whether relevant upstream kinases were activated by insulin in U937 macrophages. Incubation with insulin increased the phosphorylation of ERK1/2 threelfold (Fig. 2A). These MAP kinases induce the expression of the transcription factors c-jun and c-fos, which bind to the AP1 site. However, despite the activation of the MAP kinases, no mRNA induction of c-jun or c-fos was observed (Table 2), making it unlikely that this...
pathway was responsible for the induction of IL-1β by insulin. This view is further supported by the finding that inhibition of the MAP kinase pathway by PD-58059 did not attenuate the insulin-dependent induction of IL-1β (Fig. 3). Incubation with insulin also resulted in a 1.5-fold increase in the phosphorylation of IKKβ and the downstream phosphorylation of IkB (Fig. 2, B and C), implying that an insulin-dependent activation of NF-κB might induce the IL-1β expression.

To test whether the insulin-dependent IKKβ activation was essential for the IL-1β induction, cells were incubated with the IKKβ-specific inhibitor TPCA-1. TPCA-1 significantly reduced the IL-1β mRNA in control cells and completely abolished both the insulin-dependent and the LPS-dependent induction of IL-1β (Fig. 3). Similarly, induction of IL-1β in U937 macrophages by LPS, which had previously been shown to induce IL-1β via an activation of NF-κB, was completely abolished (not shown).

The expression of a number of additional proinflammatory or chemotactic cytokines and chemokines was also induced in U937 macrophages by insulin (Table 2). Among others, the IL-6-type cytokine oncostatin M (OSM) and the chemotactic cytokine IL-8 were significantly induced after 24-h insulin treatment. Similarly, insulin induced the expression of enzymes involved in the prostaglandin E2 synthesis, in particular the microsomal PGE synthase 1 (Table 2).

Since in a next step it was intended to test the impact of the cell culture supernatants of U937 macrophages on insulin signaling in hepatocytes, it was first tested whether insulin itself could induce cytokine production in hepatocyte cultures. At variance with such a hypothesis, insulin had no impact on the expression of IL-1β, IL-6, or OSM in hepatocytes (not shown).

Table 2. Insulin-dependent induction of genes in U937 macrophages

<table>
<thead>
<tr>
<th>Gene</th>
<th>mRNA Expression Level (AU)</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>IL-1β</td>
<td>1.00 ± 0.11</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.00 ± 0.11</td>
</tr>
<tr>
<td>CCL2</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.00 ± 0.22</td>
</tr>
<tr>
<td>OSM</td>
<td>1.00 ± 0.14</td>
</tr>
<tr>
<td>mPGES1</td>
<td>1.00 ± 0.08</td>
</tr>
<tr>
<td>COX2</td>
<td>1.00 ± 0.15</td>
</tr>
<tr>
<td>c-Jun</td>
<td>1.00 ± 0.12</td>
</tr>
<tr>
<td>TNFα</td>
<td>1.00 ± 0.12</td>
</tr>
<tr>
<td>Hmox1</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>TLR4</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>TLR2</td>
<td>1.00 ± 0.10</td>
</tr>
<tr>
<td>Srcn1</td>
<td>1.00 ± 0.09</td>
</tr>
<tr>
<td>NQO1</td>
<td>1.00 ± 0.18</td>
</tr>
<tr>
<td>c-Fos</td>
<td>1.00 ± 0.08</td>
</tr>
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</table>

Values are means ± SE of 4–31 independent experiments. U937 cells were differentiated into macrophages, as detailed in MATERIALS AND METHODS. U937 macrophages were then stimulated with 100 nM insulin for 24 h. The mRNA levels of the genes involving cytokines, chemokines, PGE2-generating enzymes, Toll-like receptors, immediate early genes (cell proliferation), and target genes in Nrf2 metabolism (oxidative stress control) were determined by RT-qPCR, with β-actin as a reference gene: CCL2, CC chemokine ligand 2 (monocyte chemoattractant protein 1); OSM, oncostatin M; mPGES1, microsomal prostaglandin E2; COX2, cyclooxygenase 2; Hmox1, heme oxygenase 1; TLR4 and -2, Toll-like receptor 4 and 2, respectively; Srcn1, sulfiredoxin 1; NQO1, NADPH/quinone oxidoreductase. Statistics: Student’s t-test for unpaired samples.

Fig. 2. Insulin-dependent activation of intracellular signaling chains in U937 macrophages. U937 cells were stimulated as detailed in the legend to Fig. 1 for 1 h. ERK1/2 (A), IKKβ (B), and IκB (C) as well as their phosphorylated counterparts (p-ERK1/2, p-IKKβ, and p-IκB, respectively) were quantified in cell lysates by densitometric analysis of Western blots using phosphospecific antibodies. Values are means ± SE of the no. of experiments indicated. Statistics: Student’s t-test for unpaired samples. *P < 0.05.

Fig. 3. Requirement of IKKβ activation for insulin-dependent induction of IL-1β in U937 macrophages. U937 cells were pretreated with 1 μM PD-98059 or 5 μM 2-(aminocarbonyl)amino)-5-(4-fluorophenyl)-3-thiophenecarboxamide (TPCA-1) for 90 min and subsequently stimulated with 100 nM insulin for 24 h. IL-1β mRNA was quantified as detailed in the legend to Fig. 1. Values are means ± SE of the no. of experiments indicated. Statistics: Student’s t-test for unpaired samples and (where appropriate) 2-way ANOVA with Tukey’s test for multiple comparison. *P < 0.05.
**Induction of insulin resistance in hepatocytes by supernatants of insulin-treated U937 macrophages.** To evaluate the potential physiological relevance of the insulin-dependent cytokine induction in U937 macrophages, the impact of the supernatants on insulin signaling in primary cultures of rat hepatocytes was investigated. The insulin-dependent induction of the key glycolytic enzyme of hepatocytes, the glucokinase, was used as readout. Insulin induced glucokinase roughly ninefold in hepatocytes kept in control medium (Fig. 4A). If hepatocytes were incubated with medium that contained 50% of the supernatants of nonstimulated U937 macrophages, glucokinase mRNA levels were similar to those found in control hepatocytes. If 100 nM insulin was added to this medium, the glucokinase was induced to a similar extent as in hepatocytes that were kept in control medium. If hepatocytes were incubated in medium that contained 50% of the supernatant of insulin-stimulated U937 macrophages, the glucokinase mRNA level was induced to about fivefold compared with control hepatocytes. This induction was elicited by the residual insulin in supernatants of insulin-treated macrophages, which was in the range of 57.68 ± 7.59 nM. However, the induction was not enhanced further by the addition of 100 nM fresh insulin. Thus, the supernatants of insulin-treated U937 macrophages inhibited the insulin-dependent induction of glucokinase, which upon maximal stimulation with a saturating concentration of insulin attained only one-half of the induction observed with the same insulin concentration in control hepatocytes.

To elucidate whether the IL-1β induction in macrophages might contribute to the inhibition of the insulin-dependent induction of glucokinase in hepatocytes, the impact of IL-1β, IL-6, and, as a negative control, LPS on the insulin-dependent induction of glucokinase was tested. In accordance with expectations, IL-1β and IL-6 significantly attenuated the insulin-dependent induction of glucokinase, whereas LPS had no impact on the insulin-dependent glucokinase induction (Fig. 4B).

Three potential mechanisms could contribute to the induction of insulin resistance in hepatocytes by the U937 macrophage supernatants: 1) IL-6-type cytokines like OSM or IL-6 itself could activate the JAK-STAT pathway and induce the expression of suppressors of cytokine signaling 3 (SOCS3), 2) IL-6-type cytokines could cause an inhibitory serine phosphorylation of IRS by an activation of MAP kinases, and 3) IL-1β could activate IKKβ and thereby cause an inhibitory serine phosphorylation of the IRS. All three hypotheses were tested.

Supernatants of insulin-treated but not nontreated U937 macrophages enhanced STAT3 phosphorylation in hepatocytes about fivefold compared with control hepatocytes (Fig. 5A). Although supernatants of nonstimulated U937 cells failed to significantly increase STAT3 phosphorylation, they caused an about fourfold induction of SOCS3 mRNA in hepatocytes, indicating that a low basal IL-6-type cytokine production in U937 macrophages was sufficient to slightly activate the cytokine receptor signal chains. This induction was more pronounced with supernatants of insulin-stimulated U937 macrophages, which caused an about eightfold induction of SOCS3 mRNA (Fig. 5B). Addition of insulin to the hepatocyte cultures enhanced neither STAT3 phosphorylation nor SOCS3 mRNA expression under any of these conditions.

IL-6-type cytokines not only activate the JAK-STAT pathway, they can also activate MAP kinases. In hepatocytes kept in control media, insulin increased ERK1/2 phosphorylation slightly but not significantly (Fig. 6A). Compared with hepatocytes that were incubated with control medium, ERK1/2 phosphorylation was already increased in hepatocytes that were incubated with medium containing supernatants of nontreated U937 macrophages, indicating that these nonstimulated macrophages already secreted mediators that can cause an ERK1/2 activation. This ERK1/2 phosphorylation was further increased slightly but significantly if 100 nM insulin was added to the medium. Compared with hepatocytes kept in media conditioned by control U937 macrophages, ERK1/2 phosphorylation was enhanced twofold if hepatocytes were incubated in media containing supernatants of insulin-stimulated U937 macrophages, indicating that insulin treatment increased the output of ERK1/2-activating cytokines from U937 macrophages. Again, this phosphorylation was enhanced further if 100 nM insulin was added to the medium.
IL-1β can activate IKKβ. Whereas supernatants of non-stimulated U937 macrophages did not affect IKKβ phosphorylation in hepatocytes, supernatants of insulin-treated U937 macrophages (almost) significantly (P = 0.053) increased IKKβ phosphorylation (Fig. 6B). To monitor the downstream effect of this activation, the expression of the reporter gene under the control of an NF-κB-responsive element was studied (Fig. 6C). In hepatocytes that were cultured in control medium, insulin did not affect the expression of the NF-κB reporter. Similarly, incubation of hepatocytes with supernatants of U937 macrophages that were cultured in the absence of insulin did not affect reporter gene expression. By contrast, supernatants of U937 macrophages cultivated in the presence of insulin significantly induced the expression of the NF-κB reporter. Unlike hepatocytes incubated in control medium, hepatocytes incubated with U937 macrophage supernatants responded with an NF-κB reporter activation to insulin, no matter whether the supernatant was derived from U937 macrophages cultured in the absence or presence of insulin.

Both activation of IKKβ and MAP kinases can result in an inactivating serine phosphorylation of IRS on different serine residues, i.e., Ser306 and Ser636, respectively. The combined serine phosphorylation was assessed with phosphoserine-specific antibodies recognizing both sites. In hepatocytes that were incubated in control medium, IRS was partly phosphorylated at Ser306 and Ser636. In these cells, insulin enhanced this phosphorylation about 2.5-fold (Fig. 6D). In hepatocytes that were incubated with supernatants of U937 macrophages, which were cultivated in the absence of insulin, the IRS serine phosphorylation was similar to that observed in insulin-treated hepatocytes in control media. However, this phosphorylation was not further stimulated by the addition of insulin. The strongest serine phosphorylation of IRS was observed in hepatocytes incubated with media from insulin-treated U937 macrophages. The ratio between phosphorylated and nonphosphorylated IRS was increased twofold compared with hepatocytes incubated with supernatants of U937 macrophages cultivated in the absence of insulin. Again, this phosphorylation was not enhanced further by the addition of insulin.

In summary, supernatants of insulin-treated U937 macrophages caused a stronger ERK1/2 and IKK activation that resulted in a stronger inhibitory IRS serine phosphorylation than supernatants of non-insulin-treated U937 macrophages, which already increased IRS serine phosphorylation compared with hepatocytes incubated in control media but apparently not to an extent that impaired insulin-dependent induction of glucokinase.

**DISCUSSION**

Hyperinsulinemia and macrophages: mechanisms. Conflicting results about the effect of insulin on cytokine production in macrophages exist. Although insulin inhibited LPS-stimulated kinase activation and TNFα production in rat alveolar macrophages (17), it enhanced the LPS-elicited IL-1α and IL-1β release from whole blood of healthy male volunteers subjected to a hyperinsulinemic euglycemic or hyperinsulinemic hyperglycemic clamp for 6 h prior to blood collection (33). In a different study, the impact on cytokine production of insulin alone was assessed. In this study, insulin increased TNFα release from cultured primary human mononuclear cells (35). In accord with the two latter studies, the expression of proinflammatory cytokines was enhanced in U937 macrophages by insulin in the current study independently of a costimulation with LPS (Fig. 1 and Table 2).

This insulin-dependent induction of IL-1β was mediated most likely via an activation of NF-κB. It has been shown previously that insulin can activate NF-κB in an insulin recep-
In accord with these previous results, phosphorylation of IKKβ and IκB was enhanced after stimulation with insulin (Fig. 2). Since the insulin-dependent induction of IL-1β was abolished by the IKK inhibitor TPCA-1 (Fig. 3), NF-κB activation appeared to be essential. The IL-1β promoter also contains an AP1 site, and insulin might have activated the transcription via an ERK1/2-dependent induction of c-jun and c-fos. However, neither transcription factor was induced (Table 2), and inhibition of MAP kinase signaling chain did not attenuate insulin-dependent IL-1β induction, making it unlikely that this pathway was of major importance.

Finally, IL-1β gene transcription can be activated by induction and activation of hypoxia-inducible factor-1α (HIF-1α) (23). It has been shown previously that insulin can also stabilize HIF-1α via an Akt-dependent inhibition of GSK-3β, which phosphorylates and thereby destabilizes HIF-1α (7). In light of the complete inhibition of the insulin-induced IL-1β expression by TPCA-1, this pathway seems to be of only minor relevance in the current setting.

IL-1β is produced as a 30.6-kDa cytosolic precursor from which the mature 17.4-kDa cytokine is cleaved by activated caspase I. The precursor can be stored and the active cytokine released subsequent to an activation of the inflammasome. Thus, the observed insulin-dependent induction of the IL-1β mRNA might prime the macrophages for a boosted IL-1β release in response to a succeeding offensive stimulus.
support of such a hypothesis, the concentration of IL-1β precursor in cell lysates was increased by insulin treatment of U937 macrophages. In light of the development of insulin resistance in the course of the metabolic syndrome, it is of note that inflammasome activation can result from overloading of macrophages with cholesterol or urate or from activation of the Toll-like receptor-4 by saturated fatty acids (18).

Similarly to IL-1β, the induction of the other cytokines and chemokines, IL-8, CCL2, IL-6, and possibly TNFα, may be caused directly by an insulin-dependent activation of NF-κB, since NF-κB regulatory elements are found in the promoters of all of these cytokines (15, 20, 36). By contrast, the OSM promoter does not seem to contain an NF-κB-binding site. Rather, AP1, C/EBP, and STAT5 appear to be major regulators (13, 16). The induction of OSM might thus be secondary to the release of the other cytokines that might act as autocrine stimuli on U937 macrophages. The observed induction of the mPGES1 (Table 2) might indicate that the production of PGE2 is also enhanced. PGE2 has previously been shown to induce OSM production in macrophages (10). A PGE2-dependent autocrine stimulation loop might not only be relevant for the induction of OSM production but could also augment the production of other cytokines. Thus, it has been shown previously that PGE2 can enhance the NF-κB-dependent release of IL-8 from human embryonic kidney-293 cells (20). Similarly, the LPS-induced release of IL-6 was enhanced by PGE2 in Kupffer cells (34). By contrast, the LPS-induced TNFα production in Kupffer cells or the CCL2 production in dendritic cells upon maturation was inhibited by PGE2 via the Gα-coupled EP2 and EP4 receptors (4, 6).

Hyperinsulinemia and macrophages: consequences. Previously, hyperinsulinemia has been shown to cause insulin resistance (27). Apart from receptor desensitization and insulin-dependent inactivation of signal chain molecules in the target cells, the insulin-induced cytokine production in macrophages observed here might contribute to the development of insulin resistance. The supernatants of insulin-treated U937 macrophages rendered primary rat hepatocytes insulin resistant (Fig. 4A). This was most likely due to the increased content of cytokines in the supernatants of insulin-treated U937 macrophages, since the respective downstream signaling cascades were activated in hepatocytes; IL-6 and OSM may be responsible for the observed STAT3 phosphorylation, SOCS3 induction, and ERK1/2 activation, whereas IL-1β may have activated IKKβ (Figs. 5 and 6). Previously, both IL-6 and IL-1β have been shown to cause insulin resistance in hepatocytes (11, 21). Although local production in resident liver macrophages in response to the very high insulin concentrations found in the portal circulation might be a relevant source, IL-1β might be formed in the abdominal adipose tissue and then act on hepatocytes since the blood from the abdominal adipose tissue drains directly into the liver.

Although the insulin-dependent activation of macrophages might be of particular importance in the liver due to the high portal insulin concentrations, it could be relevant in the initiation of the inflammation in adipose tissue observed in insulin-resistant obese patients. Thus, as has been shown recently, insulin-dependent induction of CCL2 (MCP-1) could be an early step in the recruitment of additional inflammatory cells into the expanded adipose tissue (24). IL-1β released from macrophages has been envisaged as major factor causing insulin resistance in adipocytes (2) and is a strong inhibitor of adipocyte differentiation (8). An insulin-induced production of IL-1β might thus contribute to the unfavorable phenotype of adipose tissue with large lipid-overloaded and insulin-resistant adipocytes found in obese patients.

In summary, the results obtained in the current study indicate that compensatory hyperinsulinemia that ensues excessive dietary carbohydrate intake or early-stage insulin resistance in overweight or obese patients may trigger macrophages to release proinflammatory cytokines like IL-1β, which in turn may render insulin target cells insulin resistant. Such a mechanism might be primarily relevant in liver, which is exposed to much higher insulin concentrations than any peripheral organ.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


J.H. and G.P.P. prepared figures; G.P.P. drafted manuscript.

J.H. and G.P.P. conceived and designed the experiments; J.H. performed the experiments; J.M., R.G.C., and A.P.K. performed experiments; J.M., R.G.C., A.P.K., and J.H. analyzed data; J.H. and G.P.P. interpreted data; J.H. and G.P.P. drafted the manuscript.


