

Overexpression of Protein Tyrosine Phosphatase 1B in HepG2 Cells Ameliorates Insulin-mediated Suppression of Apolipoprotein B mRNA Translation Via its Untranslated Regions

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Abstract

Background: The hepatic secretion of apolipoprotein B (apoB), containing lipoproteins, is known to be regulated by insulin, and the overproduction of these atherogenic lipoproteins occurs in insulin-resistant states. Protein tyrosine phosphatase 1B (PTP-1B) is a key regulator of hepatic insulin signaling and is also upregulated in insulin resistance. We aimed to investigate the role of PTP-1B in regulating apoB mRNA translational efficiency mediated by 5'/3' untranslated regions (UTRs) under conditions of insulin stimulation.

Methods: Human hepatoma HepG2 cells were transfected with a vector carrying the firefly luciferase reporter gene and either a chimeric apoB mRNA encoding the 5'/3' untranslated region (5'LUC3'-pGL3) or a null sequence of length equivalent to apoB 5' UTR (LUC-pGL3). The transfected cells were then infected with adenovirus carrying the mouse PTP-1B gene (AdPTP1B) in the absence or presence of insulin, and the cellular luciferase activity was determined. The RNA extracts from cells were transfected with constructs carrying 5'/3' apoB UTR, or a null sequence was also translated in vitro in a rabbit reticulocyte translation system.

Results: The luciferase activity of the cells transfected with constructs containing the apoB UTR sequences (5'LUC3') was significantly higher than that of the control constructs carrying a null sequence ($p < 0.01$, $n = 12$). Similar results were observed following in vitro translation studies showing a significantly higher expression of the 5'/3' UTR constructs ($p < 0.001$, $n = 6$). Treatment with 100 nM insulin led to a significant reduction in the luciferase activity of the constructs carrying apoB 5'/3' UTR ($p < 0.0001$, $n = 12$). The down regulation of the apoB mRNA translation mediated by insulin was mediated by the apoB 5'/3' UTR sequences since insulin did not affect the control constructs containing a null sequence. The infection of HepG2 cells expressing 5'LUC3' or control constructs with AdPTP-1B attenuated the inhibitory effect of insulin and led to higher levels of luciferase activity compared to the Ad β -gal infected control cells ($p < 0.05$, $n = 12$). However, the activity was lower than that in the control cells infected with 5'LUC3'-pGL3 but not treated with insulin ($p < 0.05$, $n = 12$).

Conclusion: Our data suggest that PTP-1B can potentially modulate apoB synthesis by blocking insulin-mediated inhibition of the apoB mRNA translation via its 5'/3' UTR sequences. We hypothesize that the PTP-1B-mediated attenuation of the insulin action can lead to the upregulation of the apoB mRNA translation and contribute to a lipoprotein overproduction in conditions such as insulin resistance.

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Introduction

Apolipoprotein B (apoB) is the major structural protein associated with very low density lipoprotein (VLDL) and low density lipoprotein (LDL) and, as such, is an important risk factor for the development of coronary artery disease. The overproduction of atherogenic apoB is thought to be a common underlying factor in many dyslipidemic conditions including those associated with insulin resistance and the metabolic syndrome.¹⁻⁶ The complex, interdependent processes that regulate the apoB lipoprotein assembly involve numerous proteins and co-factors that facilitate translation, lipidation, folding, and quality control. The inhibition of a number of these co-factors in HepG2 cells leads to posttranslational degradation of apoB via the ubiquitin-proteasome pathway, as well as other non-proteasomal pathways.⁷

Although the regulation of apoB biogenesis is thought to occur largely posttranslationally, there is also evidence that regulation may begin at the translational level.^{8,9} Insulin, thyroid hormone, and the microsomal triglyceride transfer protein (MTP) have all been reported to be involved in the translational control of apoB.¹⁰⁻¹³ The effect of insulin is believed to occur via a post-transcriptional mechanism, since the apoB mRNA level remains unaltered by insulin.¹⁴ Studies in primary rat hepatocytes have suggested that insulin decreases the apoB secretion through an increased post-translational degradation as well as a decreased synthetic rate.⁸ Despite these observations, the precise molecular mechanisms mediating the translational control of apoB remain incompletely understood.

Recent evidence based on both experimental data and thermodynamic modeling suggests that the translational control of apoB mRNA may be governed by the structural properties of the 3' and 5' UTR regions.¹⁵ ApoB mRNA is 14121 nucleotides long, and its 5' and 3'UTR are composed of 76% GC r-nucleotides that possess a high potential for forming a stable secondary structure.¹⁰ An investigation into the 5' and 3' UTR sequences of apoB mRNA revealed RNA elements with the potential to form a stable secondary structure which in turn may mediate the translational control of apoB mRNA.¹⁵ The present study investigated the role of the UTR in the regulation of the apoB mRNA translation first by analyzing the apoB UTR sequences using Mfold, a program used to predict the RNA secondary structure. The Mfold analysis revealed hairpin-like elements within the 5' and 3' UTRs of apoB mRNA with the potential to form a stable secondary structure. Chimeric mRNAs, containing the 5' and/or 3' apoB UTRs linked to an LUC reporter gene or the apoB15 sequence, were employed to investigate the biological activity of these UTR motifs.¹⁵ The data suggested that the potential cis-trans interactions of these motifs with putative RNA binding proteins/translational factors are likely to govern the apoB mRNA translation and protein synthesis and may play an important role in the dysregulation of the atherogenic lipoprotein production in dyslipidemic states.

Protein tyrosine phosphatase 1B (PTP-1B) is a phosphatase involved in the dephosphorylation of the key components of the insulin signaling cascade including the insulin receptor and IRS family of proteins.¹⁶⁻²⁰ The overexpression of this protein is believed to play a role in insulin resistance,^{21,22} and PTP-1B gene polymorphisms have been linked to the development of type 2 diabetes.²³ Interestingly, in PTP-1B knockout mice, there was a decrease in the plasma triglyceride levels as well as resistance to fat-induced insulin resistance. More recently, our laboratory has reported a direct correlation between PTP-1B expression and hepatic apoB production.²⁴ PTP-1B knockout mice were found to be resistant to a high-fructose diet, demonstrating significantly lower plasma triglyceride and apoB levels. The adenoviral-mediated overexpression of PTP-1B led to a significant increase in the apoB secretion in the cultured hepatocytes. Overall, the available data suggest a potential role for PTP-1B in the regulation of the hepatic apoB production.

In light of these observations, we investigated the possible role of PTP-1B in regulating the translational control of apoB mRNA. We employed the adenoviral-mediated overexpression of PTP-1B in HepG2 cells to determine whether PTP-1B could exert an effect on the translation of chimeric luciferase reporter constructs containing 5' and 3' UTR sequences under insulin stimulated conditions.

Methods

Cell culture HepG2 cells (American Type Culture Collection, ATCC8065) were maintained in an alpha-modification of Eagle's minimum essential medium (α -MEM) with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C in 5% CO₂.

DNA constructs The 5'/3' UTRs were generated by PCR using apoB100 cDNA as template (kindly provided by Dr. Zemin Yao, University of Ottawa Heart Institute). The UTRs were cloned into the eukaryotic vector pGL3 (Promega, Madison, WI), containing SV40 promoter and enhancer sequences and the entire sequence encoding the firefly luciferase (FF-LUC) reporter gene. The 5' UTR and 3' UTR were cloned upstream and downstream of the LUC coding sequence, respectively. A control construct was made by using a null sequence of equivalent length to the apoB 5' UTR (128 base pairs) and was cloned upstream of the LUC gene into pGL3 vector to ensure that the observed effects were due to the presence of the specific apoB UTR sequence. The transfection efficiency was monitored by using a pRL-TK vector (Promega). This vector contains the Renilla luciferase gene (R-LUC) with the HSV-thymidine kinase and is suitable to use as control because it provides neutral constitutive expression of the renilla luciferase control vector.

In vitro translation assay – Cell lysates were prepared from HepG2 cells transfected with either 5'LUC3'-pGL3 or LUC-



pGL3 and treated for 18 hours with 100 nM insulin 48 hours following transfection. The Flexirabbit reticulocyte lysate kit (Promega) was used according to the manufacturers' instructions for in vitro translation.

Transfection of DNA constructs and viral transduction
The PTP-1B adenovirus (AdPTP1Bm) was constructed as described.²⁴ 4×10^6 cells per well were seeded onto collagen-coated 6-well plates. The cells were allowed to attach to the plate overnight, and the transfection experiments were carried out at 70-80% confluency. 0.4 μ g per well of each DNA construct was transfected using the Lipofectamine Plus Kit (Life Technologies, Bethesda, MD) for 4 hours. To normalize the transfection efficiency, the cells were co-transfected with 0.4 μ g/well of the pRL-TK vector (Promega), which contained the renilla luciferase gene and the HSV-thymidine kinase promoter under the same conditions. The promoter is suitable to use as it provides the neutral constitutive expression of the renilla luciferase control vector. Following transfection, the media was replaced with complete α -MEM, and the cells were maintained for 48 hours. The cells were then harvested, cell extracts prepared, and luciferase assay was carried out by using the Dual-Luciferase Reporter Assay System (Promega). A ratio of firefly to renilla luciferase activity was calculated for each sample to normalize differences in the cell number and transfection efficiency. All the experiments, including DNA transfection and enzyme assays, were performed at least in triplicate.

Results

Effect of insulin on the expression of chimeric luciferase reporter constructs containing the 5'/3' untranslated regions of apoB mRNA

Figure 1 shows a schematic diagram of the constructs used in this study. The first construct encodes the fire fly luciferase gene (FF-LUC gene) flanked by the 5' and 3' UTR regions of the apoB gene (Figure 1A). A null sequence of equivalent length to the apoB 5' UTR was cloned upstream of the FF-LUC gene and was used as a negative control (Figure 1B). Transfection efficiency was monitored using the renilla luciferase coding region (R-LUC gene) encoding the HSV thymidine kinase promoter (Figure 1C).

We first determined whether insulin could affect the luciferase activity in HepG2 cells transfected with the 5'LUC3'-pGL3 apoB-luciferase by performing an insulin dose-response experiment (0-300 nM insulin). Figure 2A shows that the luciferase activity of 5'LUC3'apoB-pGL3 luciferase was reduced in a dose-dependent manner and was inhibited in the presence of 25 nM insulin ($p < 0.05$) and up to 300 nM insulin ($p < 0.05$). Under basal conditions (no insulin), the luciferase activity of the cells transfected with constructs containing the 5'/3'UTR of apoB (5'LUC3'-pGL3) was considerably higher than that in the cells transfected with

control constructs containing no UTR sequences (LUC-pGL3) (~4.5 fold higher, $p < 0.001$, $n = 12$) (Figure 2B). In the presence of 100 nM insulin, the luciferase activity of the cells transfected with constructs containing the 5'/3'UTR of apoB (5'LUC3'-pGL3) was significantly decreased by over 50% ($p < 0.05$) compared to that in the absence of insulin (Figure 2B). No changes in the luciferase activity was observed in the control cells transfected with the control LUC-pGL3 when treated with insulin (data not shown), suggesting that the effect of insulin on the inhibition of the luciferase activity of the 5'LUC3'-pGL3 apoB-luciferase was specific to the constructs carrying the 5'/3'UTR sequences.

In vitro translation experiments in a rabbit reticulocyte translation system were also performed by using the RNA extracts from the cells transfected with 5'LUC3'-pGL3 apoB-luciferase or control LUC-pGL3 carrying a null UTR sequence. A significantly increased luciferase activity was observed with 5'LUC3'-pGL3 (~4.2 fold, $p < 0.01$, $n = 12$) compared to that of the null-LUC-pGL3 (Figure 2C). Interestingly, the in vitro translation of the RNA isolated from the insulin-treated HepG2 cells transfected with 5'LUC3'-pGL3 showed a significantly lower luciferase activity compared to the activity of the RNA isolated from the same cells but in the absence of insulin (~40%, $p < 0.01$, $n = 12$) (Figure 2C).

Insulin did not have any significant inhibitory effect on the translation of RNA isolated from the cells transfected with the LUC-pGL3 control construct (data not shown). Overexpression of protein tyrosine phosphatase 1B (PTP-1B) partially reversed the negative effect of insulin on 5'/3' UTR of apoB. We also investigated the effect of PTP-1B on the translation of chimeric luciferase reporter constructs carrying the 5'/3' UTR of apoB mRNA. An adenovirus carrying the mouse PTP1B gene (AdPTP1Bm)²⁴ as well as a control adenovirus (Ad β -gal, controladenovirus, in which the β -galactosidase gene had been cloned) was employed.

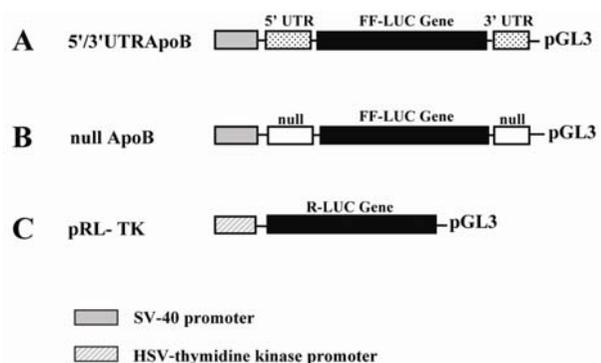


Figure 1. Schematic diagram of 5'/3' UTR apoB and control constructs. A) 5'LUC3'-pGL3 apoB-luciferase construct encodes 5' UTR and 3' UTR sequences cloned upstream and downstream of the FF-LUC gene, respectively. B) LUC-pGL3 control construct apoB-null construct in which a null sequence of equivalent length to the apoB 5' UTR was cloned upstream of the FF-LUC gene. C) R-LUC gene cloned downstream of the HSV thymidine kinase promoter was used to assess transfection efficiency.

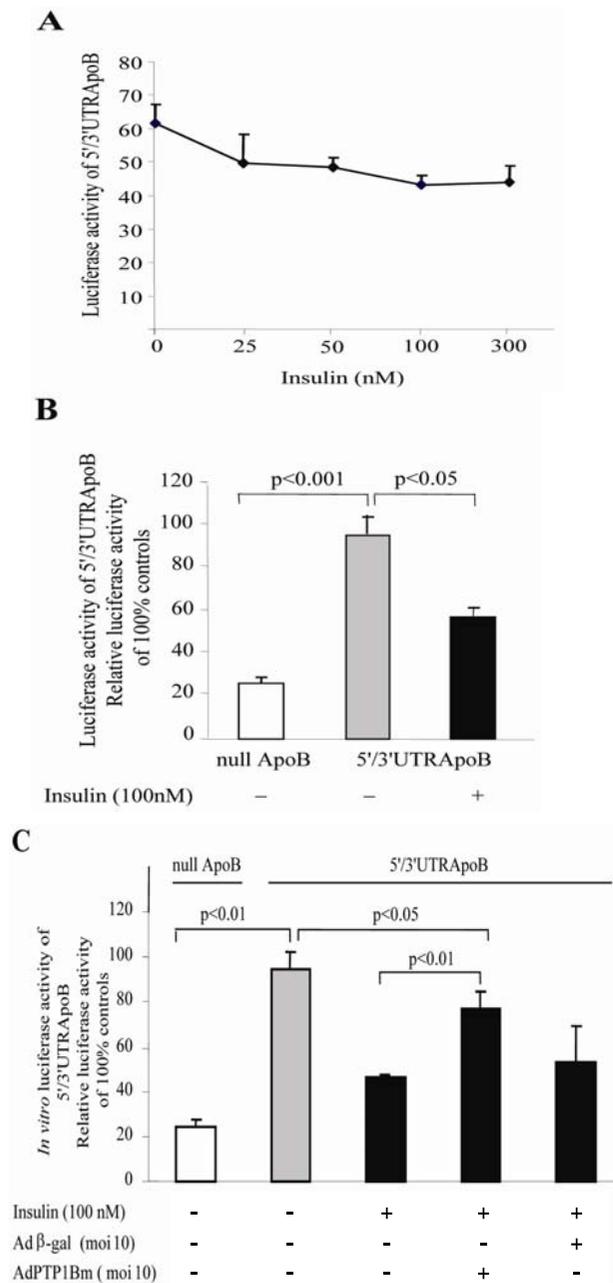


Figure 2. Effect of insulin on the expression of chimeric luciferase reporter constructs containing the 5'/3' untranslated regions of apoB mRNA. Panel A) LUC activity of 5'/3' UTR apoB constructs is suppressed by insulin in a dose-dependent manner. The suppression effect of increasing dose of insulin on the apoB5'/3' UTR-luciferase constructs in HepG2 cells. Each experiment was performed in triplicate, results are represented as mean \pm S.E., n=6. Panel B) LUC activity of 5'/3' UTR apoB constructs is suppressed by insulin. The suppression effect of 100 nM of insulin on the apoB5'/3' UTR-luciferase constructs was examined in HepG2 cells. Each experiment was performed in triplicate, results are represented as mean \pm S.E., n=12. Panel C) In vitro translation of apoB5'/3' UTR from HepG2 cell lysate. RNA isolated from HepG2 cells transfected with either the 5'LUC3'-pGL3 apoB-luciferase construct or LUC-pGL3 control construct and treated overnight with 100 nM insulin 48 hours following transfection were incubated with rabbit reticulocyte in vitro translation system. Results are means \pm S.E., n=6

The construction of these adenoviruses has been previously described.²⁴ HepG2 cells were infected with AdPTP1B or Adβ-gal viruses 30 minutes after being transfected with 5'LUC3'-pGL3 apoB-luciferase or LUC-pGL3 control. As shown in Figure 3A, the infection of HepG2 cells with AdPTP1B led to a significantly higher protein mass of PTP-1B (5.4 fold \pm 0.45, p<0.01) based on an immunoblot analysis of the HepG2 cells compared with that in the control cells infected with Adβ-gal encoding the β-galactosidase gene.

Once the overexpression of PTP-1B was confirmed under these conditions, the HepG2 cells were transfected with 5'LUC3'-pGL3

apoB-luciferase or LUC-pGL3 control construct and then infected with either AdPTP1B or Adβ-gal adenovirus. The cells were also treated with or without 100 nM insulin for 16 hours. As shown in Figure 3B, in the absence of insulin, no changes in the luciferase activity were observed in cells transfected with 5'LUC3'-pGL3 and infected with either AdPTP-1B or Adβ-gal. Interestingly, when the HepG2 cells transfected with 5'LUC3'-pGL3 and infected with the AdPTP-1B were treated with insulin, there was a significant increase in the level of the luciferase activity (Figure 3B) (an increase of ~35%, p<0.05, n=12, compared to that of the control cells infected with Adβ-gal).

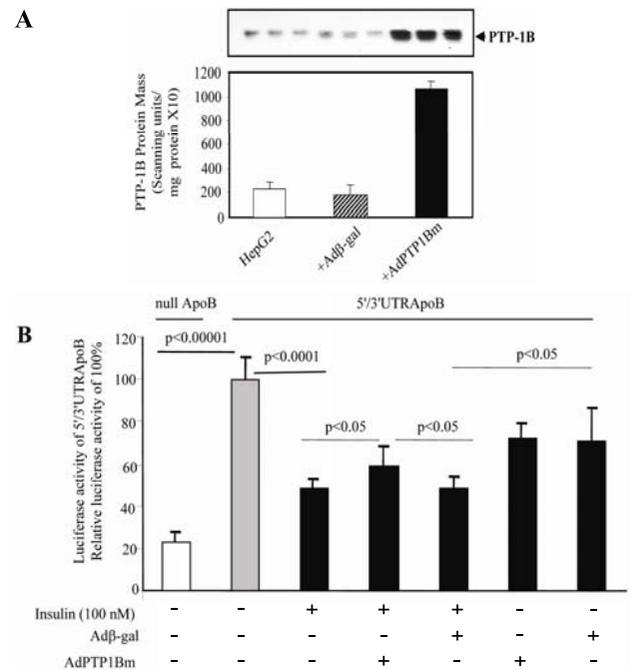


Figure 3. Overexpression of protein tyrosine phosphatase 1B (PTP-1B) partially reversed the negative effect of insulin on 5'/3' UTR of apoB. Panel A) Adenovirus-mediated overexpression of PTP1B in HepG2 cells. Immunoblot analysis of HepG2 cells transfected with empty vector, control adenovirus encoding β-galactosidase (Adβ-gal), or adenovirus encoding the mouse PTP1B mRNA (AdPTP1Bm). Results are means \pm S.E., n=3. Panel B) PTP-1B overexpression impedes the inhibitory effect of insulin luciferase activity of apoB 5'/3' UTR HepG2 cells transfected with 5'LUC3'-pGL3 apoB-luciferase construct or LUC-pGL3 control construct were infected with PTP-1B adenovirus. Results are means \pm SE, n=12



This suggests that the overexpression of PTP-1B can block the negative effect of insulin on the expression of constructs carrying the apoB-5'/3'UTR. Importantly, the luciferase activity of cells transfected with both 5'LUC3'-pGL3 and AdPTP-1B was still significantly lower than that in the cells under basal conditions (not treated with insulin) ($p < 0.05$, $n = 12$), suggesting that the overexpression of PTP-1B only partially reversed the negative effect of insulin on the expression of 5'LUC3'-pGL3. The presence or absence of insulin had no effect on the luciferase activity in cells transfected with the control construct under the same conditions. Finally, the luciferase activity in the cells transfected with 5'LUC3'-pGL3 and infected with either AdPTP-1B or Ad β -gal was tested in the absence and presence of insulin in order to ensure the luciferase activity observed was not an artifact of the transfection itself. No significant increase in the luciferase activity was observed under any of these conditions (Figure 4).

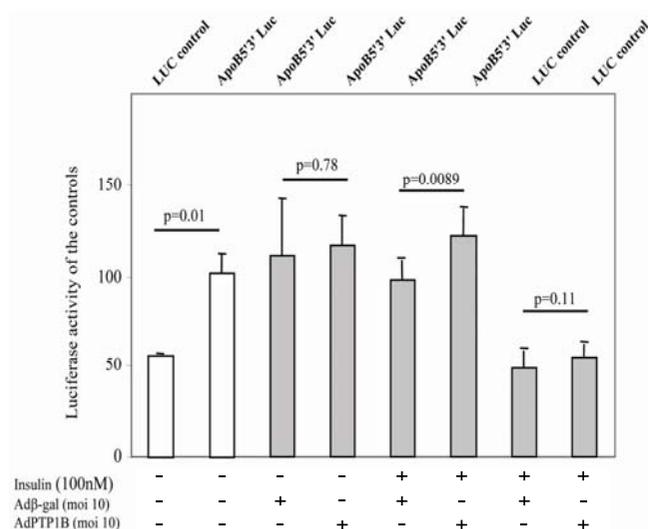


Figure 4. Insulin does not affect control luciferase reporter constructs over expressing protein tyrosine phosphatase 1B (PTP-1B). HepG2 cells transfected with 5'LUC3'-pGL3 apoB-luciferase construct or LUC-pGL3 control construct were infected with control adenovirus β -galactosidase (Ad β -gal), or adenovirus encoding the mouse PTP-1B mRNA (AdPTP1Bm) and treated overnight with 100 nM insulin 48 hours following transfection.

Discussion

It has previously been reported that insulin has a suppressive effect on the hepatic apoB assembly and secretion; however, the precise mechanism of regulation remains incompletely understood.⁸ Recent evidence implicates sequence and structural elements within the UTR sequences of apoB mRNA in the translational control and apoB synthesis.¹⁵ In a previous study, 5' and 3' apoB UTR sequences were cloned into a eukaryotic expression vector pGL3 upstream and downstream of the firefly luciferase reporter gene. The UTR sequences, and particularly the 5' UTR, had a

significant stimulatory effect on the expression and in vitro translation of the reporter construct.¹⁵ In the present study, we demonstrate that the UTR-stimulated increase in the translational efficiency can be significantly ameliorated following the insulin treatment of HepG2 cells. The insulin-mediated down regulation of the reporter expression appeared to be mediated by the 5'/3' UTR sequences since the cells expressing the control constructs lacking the UTR sequences were unresponsive to insulin exposure. In vitro translation experiments using a reticulocyte lysate system confirmed that the effects observed in the cell transfection studies were translational in nature. Interestingly, this down regulation was observed despite a global increase in total mRNA and protein abundance due to the widely-recognized anabolic effect of insulin (data not shown).

Our results show the importance of the apoB-UTR sequences in the mRNA translation by comparison with several other studies; furthermore, our findings suggest that the secondary structure and sequence of the 5' and 3' UTRs of mRNA are critical to the translational control. The UTRs within pre-proinsulin mRNA play crucial roles in regulating the insulin production and, therefore, glucose homeostasis by regulating the translation and the stability of the preproinsulin mRNA.^{25,26} The 3'UTR of the PTP-1B gene is associated with several features of insulin resistance.²⁷ Studies have generally shown that highly structured 5' UTR sequences tend to inhibit efficient translation.^{10,28} Within the 5' UTR, there are two GC boxes located at positions -20 and -81, and upstream of the translational start site, and between these two GC boxes is a GAGGCC doublet; the role of such elements in the mRNA translation is currently unknown. The 3' UTR of apoB mRNA also has the sequence elements such as AUUUA and AUUUUUA sequence elements, and AU rich regions are known to play roles in the mRNA stability.²⁹ These RNA elements within the 5' and 3' UTR sequences of apoB mRNA have the potential to form a stable secondary structure which would be expected to hinder the translation of apoB mRNA. We have, however, observed a significant upregulation of the mRNA translation following the insertion of the UTR sequences.¹⁵ This suggests the unique role of apoB UTR sequences (particularly 5' UTR) in stimulating the apoB mRNA translation.

The structure of a eukaryotic mRNA showing different types of UTR-specific regulatory elements involved in the posttranscriptional regulation of gene expression has been shown.³⁰ Unlike DNA-mediated regulatory signals, whose activity is essentially mediated by their primary structure, the biological activity of regulatory patterns acting at the RNA level relies on a combination of primary and secondary structure elements assembled in a consensus structure generally recognized by specific RNA-binding proteins. Several studies suggest that the secondary structure and sequence of the 5' and 3' UTR mediate the translational control. These structural features may be a potential binding site for RNA-binding proteins; the interaction of cis-elements

with trans-acting factors may modulate the translation or alter the stability of the message.³¹ An analysis of the apoB UTR sequences using the Mfold, a program used to predict the RNA secondary structure, revealed hairpin-like elements with the potential to form a stable secondary structure within the 5' and 3' UTR regions of apoB mRNA.¹⁵ Chimeric mRNAs containing the 5' and/or 3' apoB UTRs linked to a LUC reporter gene or the apoB15 sequence were employed to investigate the biological activity of these UTR motifs.¹⁵ The data suggest that the putative 5' UTR motifs are important for an optimal translation of the apoB message, whereas the presence of the 3' UTR appears to attenuate a wild-type expression. The potential cis/trans interactions of these motifs with the putative RNA binding proteins/translational factors are likely to govern the apoB mRNA translation and protein synthesis and may play an important role in the dysregulation of the atherogenic lipoprotein production in dyslipidemic states. More recently, we have identified a 110- kDa insulin-sensitive factor that binds to the 5' UTR and regulates the apoB mRNA translation. Insulin-mediated alterations in the binding of this factor to the 5'UTR appear to modulate the cis/trans interactions at the 5' UTR and lead to alterations in the rate of the apoB synthesis (data not shown).

Early studies in the 1990s suggested the translational control of apoB mRNA by insulin and thyroid hormone in HepG2 cells.^{13,14} Studies in primary rat hepatocytes have also shown that insulin suppresses the apoB secretion in part by stimulating the degradation of freshly translated apoB and also by reducing the apoB synthesis. It has been suggested that a reduced apoB synthesis is a result of a decreased translational efficiency.⁸ Additional studies in streptozotocin-induced diabetic rats have provided further evidence of the apoB mRNA translational control. A decreased apoB synthesis observed in primary hepatocyte cultures derived from these diabetic rats was believed to be due to a reduced translational efficiency. This was found to be a result of impaired or slowed translation rates as determined by ribosome transit studies.¹² Studies in cultured human fetal intestinal cells have also suggested that the insulin-mediated suppression of the apoB secretion may be mediated by co- and posttranslational modulation including mRNA translation.³² A more recent study in HepG2 cells found that the apoB synthesis decreased in response to treatment with CP-10447, an inhibitor of microsomal triglyceride transfer protein (MTP).¹¹ The decrease was attributed to a translational effect as the apoB mRNA levels remained unchanged in response to the MTP inhibitor. The authors postulated that the decrease in the apoB translation was due to delayed polypeptide elongation rates as determined by synchronization studies with puromycin and by ribosome transit studies.¹¹ Overall, these studies suggest that the apoB synthesis may be regulated at the level of translation by insulin. However, the molecular mechanisms or factors which mediate the translational control of apoB mRNA by insulin have not been elucidated.

Insulin signaling can be attenuated by the activity of protein

tyrosine phosphatases (PTPases) which dephosphorylate the insulin receptor, IRS-1, IRS-2, and the docking protein, Shc, leading to the modulation of insulin action.³³ PTPases constitute a very large super family of enzymes.^{20,34} Among these, PTP-1B is widely expressed in insulin sensitive tissues (liver, fat, and muscle),³⁵ can efficiently dephosphorylate the insulin receptor in vitro,^{19,36} and induce the down regulation of IRS-1 and PI-3 kinase activity.¹⁷ Increased PTP-1B has been associated with insulin resistance induced by an exposure to high glucose levels,³⁷ and such insulin resistance can be reversed by the normalization of the PTP-1B level and activity.³⁸ PTP-1B knockout mice exhibit improved insulin sensitivity on a high fat diet and increased insulin-induced insulin receptor and IRS-1 tyrosine phosphorylation.^{39,40} Interestingly, PTP-1B knockout mice were resistant to diet-induced obesity and did not develop hypertriglyceridemia on a high fat diet.³⁹ In addition, a single nucleotide polymorphism within the PTP-1B gene in humans has been shown to correlate with protection from type 2 diabetes.⁴¹ Overall, there is strong evidence suggesting that PTP-1B can modulate insulin signaling, and may thus play an important role in the pathogenesis of insulin resistance and possibly metabolic dyslipidemia.

It was of interest, therefore, to study the potential effect of PTP-1B on the suppression of the apoB mRNA translation by insulin. To study the effect of PTP-1B on the translation efficacy of insulin-treated HepG2 cells transfected with the apoB 5'/3' UTR construct, we used recombinant adenoviruses that overexpress the mouse PTP-1B gene (AdPTP1B). An adenovirus in which the β -galactosidase gene had been cloned (Ad β -gal) was also used as negative control. The overexpression of PTP-1B has recently been shown to lead to the down regulation of insulin signaling in HepG2 cells and induce the apoB100 overproduction.²⁴ In the present report, we provide evidence that a PTP-1B overexpression could partially block the insulin-mediated suppression of the expression of luciferase reporter constructs carrying 5'/3' UTR sequences. This appears to be direct evidence for an effect of PTP-1B on the translational control of mRNA translation via the 5'/3'UTR sequences. The PTP-1B overexpression was, however, unable to completely restore the translational efficiency of apoB mRNA in the presence of insulin.

Conclusion

We postulate that the PTP-1B overexpression attenuates insulin signaling transduction and thus leads to at least a partial block in the insulin-mediated suppression of the apoB mRNA translation. It is important to note, however, that our conclusions are based on the use of reporter constructs and not in the context of a complete apoB mRNA coding sequence and will need further confirmation in future studies.



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