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Inhibition of Protein Tyrosine Phosphatase-1B with Antisense Oligonucleotides Improves Insulin Sensitivity and Increases Adiponectin Concentrations in Monkeys

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Protein tyrosine phosphatase (PTP)-1B antagonizes insulin signaling and is a potential therapeutic target for insulin resistance associated with obesity and type 2 diabetes. To date, studies of PTP-1B have been limited by the availability of specific antagonists; however, treatment of rodents with antisense oligonucleotides (ASOs) directed against PTP-1B improves insulin sensitivity, inhibits lipogenic gene expression, and reduces triglyceride accumulation in liver and adipose tissue. Here we investigated ASO-mediated PTP-1B inhibition in primates. First, PTP-1B ASO (ISIS 113715) dosedependently inhibited PTP-1B mRNA and protein expression in cultured monkey hepatocytes. Subcutaneous administration of ISIS 113715 reduced PTP-1B mRNA expression in liver and adipose tissue of normal-weight monkeys by 40-50% and improved insulin sensitivity during an iv glucose tolerance test (IVGTT). In obese, insulin-resistant rhesus monkeys, treatment with 20 mg/kg ISIS 113715 for 4 wk reduced fasting concentrations of insulin and glucose and reduced insulin responses during an IVGTT. In these animals, adiponectin concentrations were also increased by 70%, most of which was an increase of high-molecular-weight oligomers. These effects were not observed in monkeys on a lower, dose-escalation regimen (1-10 mg/kg over 9 wk). Overall, the increase of adiponectin concentrations during ISIS 113715 treatment was correlated with the lowering of insulin responses during IVGTT (r = -0.47, P = 0.042). These results indicate that inhibition of PTP-1B with ASOs such as ISIS 113715 may be a viable approach for the treatment and prevention of obesity-associated insulin resistance and type 2 diabetes because they potently increase adiponectin concentrations in addition to improving insulin sensitivity. (Endocrinology 150: 1670-1679, 2009)

Protein tyrosine phosphatases (PTPs) reduce the activation state of the insulin receptor kinase and thereby inhibit postreceptor signaling in insulin-responsive tissues such as liver, muscle, and adipose (1). PTP activity in skeletal muscle and adipose tissue is increased in obesity and insulin resistance and is reduced after weight loss (2, 3). Of the PTPs, PTP-1B has gen-

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erated a great deal of interest as a potential therapeutic target for insulin resistance and obesity, as in contrast to their wild-type littermates, PTP-1B-null mice are resistant to these conditions when placed on a high-fat diet (4). The lean phenotype in PTP-1B-deficient mice is due to increased basal metabolic rate and energy expenditure (5) as well as increased sensitivity to the ef-

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Abbreviations: ASO, Antisense oligonucleotide; AUC, area under the curve; BUN, blood urea nitrogen; CI, confidence interval; CNPRC, California National Primate Research Center; HMW, high molecular weight; IACUC, Institutional Animal Care and Use Committee; IVGTT, iv glucose tolerance test; PEPCK, phosphoenolpyruvate carboxykinase; PPAR, peroxisome proliferator-activated receptor; PTP, protein tyrosine phosphatase; SREBP-1c, sterol response element binding protein-1; TC-PTPase, T cell phosphatase.

fects of exogenous leptin to reduce food intake and body weight (6). *In vitro*, overexpression of PTP-1B in cultured cells decreases insulin-stimulated tyrosyl phosphorylation of insulin receptor and insulin receptor substrate-1 (7), whereas application of neutralizing antibodies directed against PTP-1B produces the opposite effects (8).

Despite these advances, however, progress toward developing antagonists of PTP-1B as therapeutics for obesity and type 2 diabetes has been hampered by several factors, including selectivity and bioavailability (9). Interestingly, an antisense oligonucleotide (ASO) directed against PTP-1B has been shown to reduce PTP-1B mRNA expression in liver and adipose tissue (but not skeletal muscle) by about 50% and to produce significant glucose-lowering effects in hyperglycemic, insulin-resistant *ob/ob* and *db/db* mice (10). PTP-1B ASO treatment of *ob/ob* mice also down-regulated genes involved in lipogenesis in the liver and adipose tissue and reduced adipose tissue triglyceride content (11, 12). These latter effects are likely due to reductions in the expression of sterol response element binding protein-1 (SREBP-1c), an insulin-induced lipogenic transcription factor expressed in liver and adipose tissue (13).

The objective of the present study was to extend our previous findings in rodents by investigating the effects of PTP-1B ASO (ISIS 113715) administration in nonhuman primates. We first established the specificity of ISIS 113715 for the monkey PTP-1B gene *in vitro*. Next, we tested the ability of ISIS 113715 to inhibit PTP-1B gene expression in insulin-sensitive tissues of normal-weight cynomolgus monkeys. Subsequently we treated two groups of obese, insulin-resistant rhesus monkeys with ISIS 113715 and examined its effects on fasting insulin and glucose concentrations as well as insulin sensitivity as assessed by iv glucose tolerance tests (IVGTTs).

Because PTP-1B deficiency in adipose tissue, resulting from either genetic ablation or ASO treatment, has been reported to interfere with insulin signaling (14) and to regulate the expression of genes involved in lipogenesis (12), we also investigated the effects of ISIS 113715 treatment on plasma adiponectin concentrations. Adiponectin is an insulin-sensitizing adipocyte-derived hormone that links adipocyte hypertrophy with insulin resistance, inflammation, and atherosclerosis (15). Plasma adiponectin concentrations are decreased in patients with metabolic and cardiovascular diseases, including obesity and type 2 diabetes (16). Adiponectin concentrations increase after weight loss (17) and treatment with thiazolidinediones (18), drugs that improve insulin sensitivity in adipose tissue (19).

Materials and Methods

Identification and characterization of ISIS 113715 as a potent antisense inhibitor of PTP-1B expression in monkeys

ISIS 113715 has been identified as the most potent antisense inhibitor of mouse, rat, and human PTP-1B mRNAs (10). ISIS 113715 is a 20-base chimeric ASO in which a ribonuclease H-sensitive stretch of 10 2'-deoxy residues is flanked on both sides with a stretch of five 2'-O-(2-methoxyethyl-ethyl) modifications, which increase binding affinity toward mRNA sequences and confer nuclease resistance (20). Conservation of the ISIS 113715 binding site in monkeys, located between nucleotides 862 and 882 of the coding sequence for PTP-1B in humans, was confirmed by PCR (data not shown).

Characterization of ISIS 113715 in monkey hepatocytes in vitro

Primary cynomolgus monkey hepatocytes were obtained from CellzDirect (Tucson, AZ) and plated onto collagen-coated plates using William's E media containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA). Cells were allowed to attach for 2 h and were then washed and incubated overnight. ASOs were prepared by diluting in serum-free William's E media containing Lipofectin (Invitrogen) at 3 μ g/ml per 100 nM oligonucleotide. The mixture was vortexed and incubated for 15 min at room temperature to allow the formation of oligonucleotide/Lipofectin complexes. Cells were washed once with serum-free William's E media and then incubated with the oligonucleotide/Lipofectin mixture for 4 h. Subsequently the oligonucleotide/Lipofectin mixture was replaced with normal serum-containing media and incubated for the indicated times.

RNA purification from monkey hepatocytes was performed using the RNeasy 96 kit from QIAGEN (Valencia, CA), as per the manufacturer's standard protocol. RNA was analyzed by quantitative RT-PCR for PTP-1B expression using the TaqMan 7700 system (Applied Biosystems, Foster City, CA). Briefly, approximately 100 ng of total RNA was analyzed by RT-PCR using the following: forward primer, 5'-GGAGTTCGAGCA-GATCGACAA-3'; reverse primer, 5'-GGCCACTCTACATGG-GAAGTC-3'; and fluorescent probe, 5'-AGCTGGGCGGCCATTTAC-CAGGAT-3', all synthesized by Integrated DNA Technologies (Coralville, IA). PTP-1B expression was then normalized to total RNA, which was quantified using a fluorescent dye (R-11691; Molecular Probes, Eugene, OR). The expression of other mRNAs was also evaluated by using RT-PCR and the following primer-probes: phosphoenolpyruvate carboxykinase (PEPCK) forward primer, 5'-TGGGCTCACCTCTGTCGAA-3'; reverse primer, 5'-TGCCCATCCGCGTCAT-3'; probe, 5'-CTGACGGAT-TCGCCCTACGTGGTG-3'; G6-P-catalytic forward primer, 5'-GGTGC-GAGCAGCCAGAAT-3'; reverse primer, 5'-AAAGAGCGTGCCCAG-GTTC-3'; probe, 5'-CACATTGACACCACACCCTTTGCCA-3'; peroxisome proliferator-activated receptor (PPAR)-y forward primer, 5'-AGCAAAGAGGTGGCCATCC-3'; reverse primer, 5'-CCTGCACAGC-CTCCACG-3'; probe, 5'-CATCTTTCAGGGCTGCCAGTTTCGC-3'; SREBP-1c forward primer, 5'-GTCCTGCGTCGAAGCTTTG-3'; reverse primer, 5'-AGGTCGAACTGTGGAGGCC-3'; probe, 5'-AGGCCG-AAGGCAGTGCAAGAGACTC-3'.

For Western blots, monkey hepatocytes were lysed in 500 µl of extraction buffer [10 mM Tris (pH 7.4), 150 mM NaCl, 2 mM EDTA, 5 mM EGTA, 1% Triton X-100, 0.5% Nonidet P-40, 1 mM sodium orthovanadate, 1 mM NaF, protease cocktail tablet (catalog no. 1697498; Roche Molecular Biochemicals, Mannheim, Germany)]. The homogenate was centrifuged at $14,000 \times g$ for 10 min at 4 C to pellet insoluble material. The supernatant was saved as the final lysate and stored at -80 C. Forty micrograms of lysate were electrophoresed through a 10% Tris-glycine gel and transferred to an Immobilon P membrane (Millipore, Billerica, MA). The membrane was blocked in 5% milk for 30 min and then incubated with 0.25 µg/ml of either monoclonal PTP-1B antibody directed against the catalytic domain (Ab-1, catalog no. PH01; Oncogene Research Products, Cambridge, MA) or T cell phosphatase (TC-PTPase) antibody (catalog no. PH03L) overnight. The membrane was washed extensively and then incubated with antimouse horseradish peroxidase secondary antibody (1:10,000; Transduction Laboratories, Lexington, KY) for 1 h. PTP-1B protein bands were visualized using ECL-Plus reagents and film (Amersham, Piscataway, NJ). PTP-1B protein bands were quantitated using ImageQuant software (Amersham).

Effects of ISIS 113715 on PTP-1B expression and glucose tolerance in normal cynomolgus monkeys

The effects of ISIS 113715 on PTP-1B expression and glucose tolerance were assessed in seven mature normal-weight cynomolgus monkeys (*Macaca fascicularis*). Four untreated normal-weight control monkeys

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were also studied. These experiments were performed at Shin Nippon Biomedical Laboratories USA, Ltd, (Everett, WA). Shin Nippon Biomedical Laboratories is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and has an Animal Welfare Assurance issued by the Office of Laboratory Animal Welfare. The protocol complied with applicable laws and regulations concerning the humane care and use of laboratory animals and was approved by the Institutional Animal Care and Use Committee (IACUC).

All monkeys underwent IVGTTs at several time points during the study (see below). Before ASO treatment, all monkeys underwent baseline IVGTTs. Monkeys in the treated group then received 3 mg/kg ISIS 113715, dissolved in PBS (pH 7.2), by sc injection. An IVGTT was then performed on the fourth day after dosing. This entire cycle was repeated with doses of 6 and 12 mg/kg over the next 2 wk, respectively. Ten days after the 12 mg/kg dose, the animals were given an additional 6 mg/kg sc injection. After another 3 d, monkeys were anesthetized with iv pentobarbital and phenytoin sodium so that samples of liver, skeletal muscle (quadriceps femoris), and abdominal sc fat could be collected. Animals were subsequently euthanized with an iv dose of pentobarbital (25 mg/kg). The tissues were assayed for PTP-1B mRNA and protein levels as described above.

Effects of ISIS 113715 on fasting glucose, insulin levels, and glucose tolerance in obese, hyperinsulinemic rhesus monkeys

To extend the results obtained in normal-weight cynomolgus monkeys, the effects of ISIS 113715 on fasting insulin levels and glucose tolerance were evaluated in 20 obese, insulin-resistant, hyperinsulinemic rhesus monkeys (*Macaca mullata*). Animals were fed standard chow *ad libitum* and were housed in the Assessment and Accreditation of Laboratory Animal Care-accredited facilities of the California National Primate Research Center (CNPRC) in accordance with standards established by the U.S. Animal Welfare Act and the Institute of Laboratory Animal Resources. The experimental protocols were approved by the IACUC at the University of California, Davis, and the CNPRC and were conducted in accordance with the guidelines of the National Research Council's Guide for the Care and Use of Laboratory Animals.

All monkeys were obese (body mass index >30 kg/m²) but normoglycemic; therefore, the primary end points in this part of the study were changes in fasting insulin and lipid concentrations. During the baseline period, blood samples were collected from overnight (16 h) fasted animals for measurements of glucose, insulin, and lipids. On a separate day than that of the fasting blood draws, a baseline IVGTT was conducted. After these baseline procedures, 15 monkeys underwent a dose-escalation regimen, which consisted of a 2-wk period of treatment with ISIS 113715 at 1 mg/kg, followed by 6 wk at 3 mg/kg, and 3 wk at 10 mg/kg. At the start of each dosing period, animals received ISIS 113715 sc (in the interscapular region) on 3 alternate days during the first week (loading dose) and once each week during each subsequent week (maintenance dose). During the dose-escalation regimen, an IVGTT was performed in the second week of treatment, 48 h after the last dose.

Five additional obese, hyperinsulinemic monkeys were treated with ISIS 113715 for 4 wk at a dose of 20 mg/kg, after a 1-wk baseline period. As above, animals received ISIS 113715 on 3 alternate days during the first week (loading dose), and once each week afterward (maintenance dose). During the second week of treatment, fasting samples were collected 48 h after dosing. An IVGTT was performed during the baseline period and during the third week, 48 h after dosing.

Because repeated tissue biopsies were prohibited by the CNPRC, analyses of PTP-1B gene expression and protein levels in target tissues could not be conducted in this part of the study.

IVGTTs

IVGTTs were performed on 16 h-fasted monkeys. All procedures in both the studies were conducted according to each institution's IACUC guidelines. Briefly, the monkeys were anesthetized with ketamine (10 mg/kg) and an iv catheter was inserted in each arm vein. Three baseline samples (3 ml) were collected from one catheter at -10, -5, and 0 min. Subsequently 600 mg/kg of 50% dextrose were administered in the contralateral catheter. Additional 3-ml blood samples were collected at 1, 3, 5, 10, 15, 20, 30, 40, and 60 min in obese monkeys and at 1, 3, 5, 10, 20, 40, and 60 min in normal-weight monkeys.

Biochemical measurements

Plasma glucose levels were measured using a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin levels were measured by RIA (Linco, St. Charles, MO). Blood urea nitrogen (BUN), bilirubin, creatinine, aspartate aminotransferase, aminotransferase, and lipid measurements were performed according to standard methods at the CNPRC Clinical Biochemistry Laboratory.

Plasma adiponectin concentrations were measured by RIA (Linco). Adiponectin oligomers were measured using an ELISA for human adiponectin (Alpco, Salem, NH). In our laboratory, the ELISA yielded values for total adiponectin that were about 2.5 times lower than those obtained using the RIA (21). However, the correlation between the two methods was very high [r = 0.94, 95% confidence interval (CI) 0.90–0.96, P < 0.0001]. The interassay coefficients of variation for total and high-molecular weight (HMW) adiponectin measured by ELISA were 9.8 and 12.9%, respectively; the intraassay coefficients of variation for total and HMW adiponectin were less than 6.9% and less than 10%, respectively.

Data analysis

The insulin response during the IVGTT was quantified as the area under the curve (AUC) between 0 and 60 min after dextrose infusion. Calculations were performed by a trapezoidal method using Excel software (Microsoft, Richmond, CA). Subsequent statistical analyses were performed using Prizm software (version 4.03; GraphPad Software, San Diego, CA). All variables were assessed for normality using the Kolmogorov-Smirnov test. Comparisons were made between control and treatment groups using paired *t* tests or one-way ANOVA, as appropriate. When variables were not normally distributed, the appropriate nonparametric test was used instead. For repeated measures with more than two time points, repeated-measures ANOVAs were used, and posttests were performed using Bonferroni's test for multiple comparisons, with the initial baseline measurements used as the reference. In all cases, the twotailed level of significance was P < 0.05.

Results

Effects of ISIS 113715 in primary monkey hepatocytes

In cultured monkey hepatocytes, ISIS 113715 treatment dosedependently reduced PTP-1B mRNA expression, with an IC₅₀ of less than 10 nM (Fig. 1A). Administering a control oligonucleotide (141923, which contained a 6-bp mismatch to the 113715 sequence) did not significantly alter PTP-1B mRNA expression. The specificity of the PTP-1B ASO was further investigated by Western blot analysis for PTP-1B and TC-PTPase, a phosphatase that has 80% homology with PTP-1B in the enzyme's catalytic domain. Although ISIS 113715 reduced PTP-1B protein levels by greater than 90%, no effects were observed on protein levels of TC-PTPase after ASO treatment (Fig. 1B). The changes in TC-PTPase mRNA expression could not be evaluated due to lack of available genomic sequence for the monkey TC-PTPase gene.

Effects of ISIS 113715 on PTP-1B expression in normalweight cynomolgus monkeys

ISIS 113715 administration reduced PTP-1B mRNA expression in both liver and adipose tissue by 40–50% but did not



FIG. 1. Treatment of primary cynomolgus hepatocytes with a PTP-1B antisense oligonucleotide, ISIS 113715, reduces PTP-1B mRNA and protein expression. A, Primary cynomolgus monkey hepatocytes were treated with either PTP-1B-ASO (ISIS 113715) or a 6-base mismatch control ASO (141923) at the indicated concentrations for 24 h. PTP-1B mRNA expression was analyzed by quantitative RT-PCR (TaqMan 7700) as described in *Materials and Methods*. PTP-1B mRNA expression was normalized to total RNA levels and is expressed as percent of the levels in untreated cells. Data are expressed as means \pm sEM, n = 4. *, *P* < 0.001 vs. the mismatch control ASO. Data are representative of three independent experiments. B, Primary cynomolgus monkey hepatocytes were treated with ISIS 113715 at a concentration of 150 nm for 72 h. PTP-1B protein expression was assessed by Western blots as described in *Materials and Methods*. Identical blots were probed for TC-PTPase expression to confirm specificity of PTP-1B reduction.

affect muscle PTP-1B expression (Fig. 2A). These findings are similar to those observed in rodent studies and are consistent with limited distribution of these compounds to muscle (22). Hepatic PTP-1B protein was also reduced by about 60% (Fig. 2B). PTP-1B protein levels in adipose tissue could not be determined due to the presence of an unidentified band of protein that migrated at the same molecular weight as PTP-1B (data not shown).

Decreased hepatic PTP-1B expression was accompanied by marked and significant reductions in the mRNA expression of two key gluconeogenic enzymes, PEPCK and glucose-6-phosphatase (Fig. 3, A and B). In abdominal sc adipose tissue, the mRNA expression of SREBP-1c was reduced to $44 \pm 6\%$ of control levels (Fig. 3C), whereas PPAR γ mRNA expression remained unchanged (Fig. 3D).



FIG. 2. Effects of ISIS 113715 on PTP-1B expression in normal cynomolgus monkey tissues. Animals were treated for 4 wk with ISIS 113715, after which tissues were harvested and evaluated for changes in PTP-1B mRNA expression (A) or protein expression (B) as described in *Materials and Methods*. Scans of representative gels are shown for reference. Protein expression in adipose tissue could not be determined due to the presence of an interfering band on the gels (data not shown). Membranes were routinely stripped and reprobed with a glyceraldehyde 3-phosphate dehydrogenase (G3PDH) antibody for normalization. Data are expressed as mean $\pm s_{\rm SM}$ and have been expressed as percent saline control (control being 100%) (n = 4 for the saline control and n = 7 for the ISIS 113715-treated group). *, *P* < 0.05.

Effects of ISIS 113715 on fasting insulin and glucose concentrations in normal-weight cynomolgus monkeys

Administration of ISIS 113715 to nonobese cynomolgus monkeys dose-dependently reduced fasting plasma insulin concentrations (Fig. 4). At the 5-wk time point, after a dose of 12 mg/kg, plasma insulin concentrations in the ASO-treated animals were about 50% lower compared with their baseline values (wk 5: 18.6 \pm 7.4 vs. baseline 33.9 \pm 6.6 μ U/ml, P < 0.05). In contrast, fasting insulin levels tended to increase in control animals over the same period (baseline: 23.8 \pm 1.4 μ U/ml vs. wk 5: 40.8 \pm 7.5 μ U/ml, P > 0.10).

The decreases in fasting insulin concentrations were not associated with changes in fasting glucose concentration in either the

treated or control groups. In the control group, the glucose levels ranged from 48 to 52 mg/dl (2.7–2.9 mmol/liter), and in the treated group, the average values ranged from 53 to 54 mg/dl (2.9–3.0 mmol/liter) throughout the study.

Effects of ISIS 113715 on glucose and insulin responses during IVGTTs in normal-weight monkeys

There were no differences between the slopes of the glucose disappearance curves, an index of glucose use, before or after ASO treatment (data not shown). There were also no apparent effects of ASO treatment on the glucose AUC or maximum glucose concentrations observed after iv glucose administration.

However, dose-dependent reductions in the AUC for insulin were observed in the treated animals. In the animals treated with the highest dose, the 60-min AUC was reduced by

> about 25% in ISIS 113715-treated animals (baseline: 12448 \pm 8047 μ U·min·ml *vs.* wk 5: 9638 \pm 6431 μ U·min·ml, *P* < 0.05). No changes in the insulin AUC were observed in the control animals (baseline: 10591 \pm 1892 μ U·min·ml *vs.* wk 5: 9992 \pm 167 μ U·min·ml, *P* > 0.05).

> An index of insulin sensitivity can be derived from the ratio of the slope of the glucose disappearance curve (from 5 to 20 min) and the AUC of insulin. At wk 5, there was a modest increase in insulin sensitivity in the ISIS 113715-treated group compared with baseline values (week 5: 2.12 ± 0.47 *vs.* baseline: 1.61 ± 0.89 , P = 0.04). However, in animals receiving saline, this index of insulin sensitivity was unchanged at wk 5 compared with baseline values (wek 5: 1.60 ± 0.42 *vs.* baseline: 1.63 ± 0.57 , P > 0.05).

Over the course of the study, no signs of illness, malaise, or other evidence of toxicity were observed. There were also no changes in

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FIG. 3. Significant changes in mRNA expression of hepatic gluconeogenic enzymes and adipocyte transcription factors in ISIS 113715-treated monkeys. Animals were treated for 4 wk with ISIS 113715, after which tissues were harvested and evaluated for changes in PEPCK (A), glucose-6-phosphatase mRNA expression (B), SREBP1c (C), and PPAR γ (D) by using RT-PCR, as described in *Materials and Methods*. Data are shown as mean \pm sEM and are expressed as percent saline control (control being 100%) (n = 4 for the saline control and n = 7 for the ISIS 113715-treated group). *, *P* < 0.05.

serum biochemistry parameters that were attributable to ASO treatment (data not shown).

Effects of ISIS 113715 on fasting insulin and glucose and responses to IVGTTs in obese, insulin-resistant rhesus monkeys

To extend the findings observed in normal-weight cynomolgus monkeys, the effects of ISIS 113715 treatment on fasting insulin and glucose levels and after iv glucose administration were investigated in obese, insulin-resistant, hyperinsulinemic rhesus monkeys. These monkeys spontaneously develop obesity accompanied by insulin resistance, which over time can progress to overt type 2 diabetes (23).

In monkeys on the low-dose/dose-escalation regimen, no significant effects on fasting insulin concentrations were observed over the course of the study (P = 0.32, ANOVA; Fig. 5A). However, in the animals receiving the higher dose of ISIS 113715 (20 mg/kg), fasting insulin concentrations were significantly reduced at the end of the dosing regimen (P = 0.040, ANOVA; Fig. 5A). The reductions in fasting insulin in this group were accompanied by modest but significant (P = 0.019) reductions of fasting glucose concentrations (Fig. 5B). In both groups, fasting glucose concentrations remained in the normal range throughout the study and were comparable with those observed in other studies of rhesus monkeys (24). Consistent with the above results, a significant reduction in insulin excursions during the IVGTT was observed in the animals receiving the 20 mg/kg dose but not in those on low-dose/dose-escalation regimen (Fig. 6). The mean insulin AUC decreased by nearly 40%, from 14,953 \pm 2,361 μ U \cdot min \cdot ml at baseline to 9,211 \pm 2,179 μ U \cdot min \cdot ml after treatment (P = 0.0008). Plasma glucose excursions during the IVGTT were unaffected.

Other effects of PTP-1B inhibition with ISIS 113715

We observed a small but significant reduction of body weight in monkeys treated with the 20 mg/kg dose of ISIS 113715 (Table 1). On average, monkeys lost 0.6 kg (95% CI 0.2-1.1 kg). In contrast, monkeys on the dose-escalation regimen gained a small amount of weight (0.9 kg, 95% CI 0.4-1.4 kg). The decrease of body weight was accompanied by a decrease of fasting leptin concentrations in the high-dose group (Table 1). Treatment with ISIS 113715 at 20 mg/kg was also associated with reductions of total and low-density lipoprotein cholesterol, of 12 and 16%, respectively (both P <0.05). In the low-dose/dose-escalation group, the only significant biochemical change observed in response to treatment was a slight but significant fall in bilirubin concentrations, which remained in the nor-

mal range. Renal and liver function, as assessed by BUN/creatinine and hepatic transaminase levels, respectively, were unchanged. No significant signs of illness, including injection site reactions, were observed.

Effects of PTP-1B inhibition on total and HMW adiponectin concentrations

Plasma adiponectin concentrations were increased by 70 \pm 22% in the monkeys receiving the 20 mg/kg dose of ISIS 113715 for 3 wk (mean 9.6 \pm 2.6 vs. 16.1 \pm 4.1 µg/ml, P = 0.045). In contrast, adiponectin was unchanged in the animals in the lowdose/dose escalation group (mean 11.2 \pm 1.0 vs. 10.4 \pm 0.9 μ g/ml, P = 0.27) (Fig. 7A). Measurement of adiponectin multimers in the high-dose group revealed that most (\sim 75%) of the increase in total adiponectin was due to an increase in HMW oligomers (Fig. 7B). A concomitant increase in the proportion of adiponectin in the HMW form $[S_A (25)]$ was also observed in the high-dose group (baseline: $0.42 \pm 0.11 vs.$ after treatment: 0.60 ± 0.07 , P = 0.038). With the exception of one monkey on the dose-escalation regimen in which the insulin AUC during the IVGTT was increased with treatment, the changes of adiponectin concentrations after ISIS 113715 were proportional to the changes of insulin AUC in all monkeys (Spearman correlation, r = 0.47, P = 0.042, Fig. 7C).



FIG. 4. Dose-response effects of ISIS 113715 on 16 h-fasted insulin levels in normal monkeys. The mean concentrations of insulin at baseline (0 mg/kg), 3, 6, and 12 mg/kg are shown as *gray squares* (n = 7). For reference, the control values at the start of study (depicted at 0 mg/kg) and at the end of treatment (depicted at 12 mg/kg) are shown as *black triangles* (n = 4). Data are shown as mean \pm SEM.

Discussion

In this study we investigated the metabolic effects of inhibiting PTP-1B expression in the tissues of nonhuman primates using an antisense oligonucleotide (ISIS 113715). Previous studies of PTP-1B inhibition with ISIS 113715 in obese hyperinsulinemic rodents found that PTP-1B ASO treatment improves insulin sensitivity, normalizes plasma glucose levels, and reduces adiposity by down-regulating genes involved in lipogenesis in both liver and adipose tissue (10, 12). To date, however, similar studies have not been performed in nonhuman primates.

In the current study, we first established that PTP-1B ASO treatment reduces PTP-1B mRNA and protein expression in cultured monkey hepatocytes. Next, we demonstrated that treatment of monkeys with sc administered ISIS 113715 reduces PTP-1B mRNA in liver and adipose tissue but not skeletal muscle. This is consistent with previous studies showing that ASOs accumulate in many tissues but are poorly distributed to muscle (26). We also demonstrated that in addition to the inhibition of hepatic PTP-1B expression, the expression of two key gluconeogenic enzymes (PEPCK and glucose-6-phosphatase) is also reduced in the livers of ASO-treated monkeys. Finally, we showed that in obese, insulin-resistant, hyperinsulinemic monkeys, administration of 20 mg/kg ISIS 113715 reduced fasting concentrations of glucose and insulin as well as insulin excursions during an IVGTT. Concentrations of adiponectin, an insulinsensitizing hormone produced by adipocytes, were also increased after 20 mg/kg ISIS 113715 treatment.

This study is thus the first to report the metabolic effects of PTP-1B inhibition in nonhuman primates. In addition to the high relevance of the model for human metabolic disease, an additional strength of the present study is that the effects of ASO administration on PTP-1B mRNA and protein expression in insulin-sensitive tissues were measured. Its main limitations were that food intake and changes in body composition were not assessed and that we did not have a formal control group for the studies of obese, insulin-resistant monkeys treated with ISIS 113715. However, the lack of effects on indices of insulin sensitivity or plasma adiponectin concentrations in the monkeys receiving lower doses of ISIS 113715 (on the dose-escalation



FIG. 5. Changes of fasting insulin and glucose concentrations in obese, insulinresistant monkeys treated with ISIS 113715. A, Fasting insulin concentrations. B, Fasting glucose concentrations. Data are shown as mean \pm sEM. *, P < 0.05 (n = 15 for the low dose/dose escalation group and n = 5 for the high dose group).

regimen) strongly suggests that the observed effects are not a result of the treatment regimen *per se* but are due to the effects of the higher dose to inhibit PTP-1B.

Most of the metabolic effects of PTP-1B ASO treatment observed in the study (*i.e.* reduced fasting insulin and glucose concentrations and an attenuated insulin response to an IVGTT) are likely due to ASO-mediated inhibition of PTP-1B expression in the livers of treated animals. In this regard, our results are consistent with those of Haj *et al.* (27), who reported that in high-fat diet-fed mice, the effects of overall PTP-1B deficiency were largely reversed by adenovirally mediated overexpression of PTP-1B in the liver. The importance of normal hepatic insulin signaling for the maintenance of glucose homeostasis in mice is well illustrated by the phenotype of liver-specific insulin receptor knockout mice, which exhibit dramatic insulin resistance, severe glucose intolerance, and an inability of insulin to suppress hepatic glucose production (28). However, we cannot exclude PTP-1B-mediated effects in extrahepatic tissues, particularly adipose



FIG. 6. Inhibition of PTP-1B with ISIS 113715 improves insulin sensitivity in obese, insulin-resistant rhesus monkeys. A, IVGTT results (insulin and glucose responses) from monkeys on the low-dose/dose-escalation regimen. B, IVGTT results (insulin and glucose responses) from monkeys on the high-dose regimen. Data are shown as mean \pm SEM (n = 15 for the low dose/dose escalation group and n = 5 for the high dose group).

tissue and the hypothalamus (see below). Interestingly, the effects of PTP-1B inhibition vary among different cell types in the liver: we have demonstrated elsewhere that a 50% reduction in PTP-1B expression in whole-liver extracts corresponds to a much greater reduction of PTP-1B expression in hepatocytes (29) than in nonparenchymal cells, although basal PTP-1B expression is much (6- to 8-fold) higher in the latter (30).

Inhibition of PTP-1B in adipose tissue may also have contributed to the improved insulin sensitivity because high-dose ISIS 113715 treatment substantially increased (by 70%) circulating concentrations of adiponectin, an insulin-sensitizing hormone produced exclusively by adipocytes (15). A relationship between PTP inhibition and increased adiponectin levels has been suggested previously: treatment of ZDF rats with a nonspecific PTP inhibitor, bis(maltolato) oxovanadium (iv), restored plasma adiponectin concentrations to levels seen in normal rats (31). In the present study, significantly increased plasma adiponectin concentrations were observed only in monkeys treated with the higher dose of ISIS 113715, and the changes of adiponectin concentrations in both the low- and high-dose monkeys were proportional to the reduction of insulin responses, measured by the insulin AUC during an IVGTT. The increase of adiponectin concentrations in the high-dose-treated monkeys was largely due to a greater amount of the HMW oligomer, changes which are preferentially associated with improved hepatic insulin sensitivity (25). Increased concentrations of HMW oligomers are commonly observed after weight loss associated with gastric bypass surgery (32) and treatment with thiazolidinediones (25). Moreover, replacement of different adiponectin multimers in adiponectin-deficient mice demonstrates that the HMW form most effectively lowers glucose concentrations (25).

The increase of adiponectin concentrations observed in response to high-dose PTP-1B ASO treatment may have been due to direct effects of PTP-1B silencing in adipocytes (resulting in improved insulin sensitivity) or secondary effects from improved hepatic insulin signaling. Evidence to support a primary effect on adipose tissue includes the reported ability of PTP-1B ASO treatment to reduce adipocyte mass in ob/ob mice via inhibition of lipogenic gene expression (12). Consistent with these findings, in the present study, we found that PTP-1B ASO treatment was associated with reduced mRNA expression of SREBP-1c, a transcription factor that regulates lipogenesis in adipocytes. Smaller adipocytes are generally more sensitive to the antilipolytic effects of insulin and as a result secrete less free fatty acids (33). Relative to larger, insulin-resistant adipocytes, smaller adipocytes also secrete lower amounts of proinflammatory cytokines (34) and more adiponectin (35, 36). Whereas we did not di-

rectly assess fat mass or adipocyte size in the present study, we did observe a small but significant reduction of body weight in the monkeys treated with the high dose (20 mg/kg). This was accompanied by a significant decrease of plasma leptin, which is consistent with a reduced adipose mass and cell size. Unlike leptin, adiponectin concentrations usually increase after weight loss (17). It is unlikely that weight loss was the most important factor responsible for the observed increase of adiponectin concentrations in the study, however, because the degree of weight loss in these monkeys (~0.6 kg or 4% of body weight) is at the lower limit of that associated with significant increases of adiponectin concentrations in humans (37, 38).

It remains to be demonstrated whether blocking PTP-1B in adipose tissue directly improves overall insulin sensitivity. PTP-1B knockout mice do not exhibit differences in insulin signaling or glucose uptake in adipose tissue relative to wild-type mice (5). In fact, investigation of glucose uptake in isolated adipocytes from PTP-1B-deficient mice indicates that the response to insulin is significantly attenuated relative to wild-type mice (14). Similarly, PTP-1B ASO treatment does not seem to affect insulin signaling in adipose tissue of *ob/ob* mice (12), although it was shown to reduce PPAR γ mRNA expression in addition to its inhibitory effects on SREBP-1. In the lean monkeys, we observed a significant decrease in SREBP-1c expression, whereas expression of PPAR γ remained unchanged. The differences in PPAR γ

| | Low dose (n = 15) | | | High dose (n = 5) | | |
|------------------------------------|-------------------|-----------------|---------------------------|-------------------|-----------------|-------|
| Parameter | End of baseline | After 10 mg/kg | Р | End of baseline | After 20 mg/kg | Р |
| Adiposity | | | | | | |
| Body weight (kg) | 15.3 ± 0.4 | 16.2 ± 0.5 | 0.016 | 14.4 ± 2.4 | 13.8 ± 2.3 | 0.002 |
| Leptin (ng/ml) | 12.6 ± 2.4 | 12.7 ± 3.0 | 0.45 ^a | 8.2 ± 1.0 | 4.9 ± 0.6 | 0.047 |
| Lipids | | | | | | |
| Total cholesterol (mmol/liter) | 4.12 ± 0.36 | 3.75 ± 0.49 | 0.24 | 4.50 ± 0.28 | 3.95 ± 0.27 | 0.029 |
| HDL cholesterol (mmol/liter) | 1.37 ± 0.16 | 1.42 ± 0.17 | 0.67 | 1.67 ± 0.16 | 1.72 ± 0.15 | 0.15 |
| LDL cholesterol (mmol/liter) | 2.02 ± 0.22 | 1.75 ± 0.26 | 0.36 | 2.18 ± 0.26 | 1.82 ± 0.17 | 0.043 |
| Triglycerides (mmol/liter) | 1.54 ± 0.58 | 1.21 ± 0.42 | 0.10 ^a | 1.42 ± 0.37 | 0.90 ± 0.13 | 0.16 |
| Cholesterol to HDL ratio | 3.9 ± 1.0 | 2.9 ± 0.3 | 0.083 ^a | 2.8 ± 0.3 | 2.3 ± 0.1 | 0.089 |
| Other biochemical data | | | | | | |
| Creatinine (μ mol/liter) | 67 ± 3 | 66 ± 3 | 0.68 ^a | 66 ± 5 | 66 ± 6 | 0.99 |
| ALT (U/liter) | 49 ± 5 | 46 ± 3 | 0.45 | 63 ± 26 | 49 ± 17 | 0.19 |
| AST (U/liter) | 25 ± 1 | 36 ± 5 | 0.11 | 29 ± 3 | 27 ± 2 | 0.49 |
| BUN (mmol/liter) | 6.4 ± 0.3 | 6.0 ± 0.3 | 0.12 | 6.5 ± 0.4 | 6.0 ± 0.6 | 0.13 |
| Total bilirubin (μ mol/liter) | 3.8 ± 0.5 | 2.5 ± 0.2 | 0.016 [∂] | 3.4 ± 0.5 | 3.4 ± 1.3 | 0.99 |

TABLE 1. Changes of body weight, lipids, and other biochemical measurements in obese, insulin-resistant rhesus monkeys treated with ISIS 113715

Data are shown as mean \pm sEM. Significant *P* values (*P* < 0.05) are highlighted in *bold*. HDL, High-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine transaminase; AST, aspartate aminotransferase.

^a Nonparametric Wilcoxon signed rank test.

expression between the studies conducted in obese mice and the present study may be explained, at least in part, by the very different models used (leptin-deficient ob/ob mice vs. lean monkeys). However, it is more important to note that the increase in adiponectin levels in nonhuman primates treated with the PTP-1B ASO was not accompanied by an increase in PPAR γ expression. Both SREBP-1c and PPAR y are positive regulators of adiponectin gene transcription (40, 41), and mice with an adipose-specific knockout of PPAR γ have reduced circulating adiponectin concentrations (42). Therefore, it is likely that other factors, perhaps related to insulin sensitivity in adipose tissue and/or liver, adipocyte size or lipid content, contribute to the increased adiponectin secretion. Other influences may include autonomic nervous system-mediated cross talk between the liver and visceral adipose tissue (43) because visceral adipose tissue is a major determinant of plasma adiponectin concentrations (44) or perhaps an as-yet-unidentified humoral factor. These possibilities should be addressed in future studies.

Lastly, although ASOs generally are not targeted to the brain (45), it is also possible that the observed effects of PTP-1B ASO treatment in the present study were due to PTP-1B inhibition in insulin receptor-containing areas of the hypothalamus, which regulate energy balance and are accessible to the systemic blood supply, such as the median eminence (46). Notably, neuronal-specific deletion of PTP-1B in mice is associated with higher adiponectin levels in addition to improved glucose homeostasis (47).

The substantial reduction of fasting insulin concentrations and marked improvement of insulin sensitivity observed in the obese, insulin-resistant, hyperinsulinemic monkeys in the current study supports the therapeutic potential for ISIS 113715 in the treatment of insulin resistance and type 2 diabetes. Furthermore, the magnitude of the decrease of fasting insulin concentrations (~40%) observed in the obese monkeys treated with the PTP-1B ASO is comparable with that observed after thiazo-

lidinedione (pioglitazone) treatment (39). While the manuscript was in preparation, the effects of ISIS 113715 were evaluated in healthy volunteers who received four doses of ISIS 113715 or a placebo (PBS) by parenteral administration in a randomized, double-blind, dose-escalation phase I trial. The efficacy of the two highest doses of ISIS 113715 (5.0 and 7.5 mg/kg) was assessed with an IVGTT. All ISIS 113715-treated volunteers (n = 6) exhibited increased insulin sensitivity compared with placebotreated subjects (n = 2), which was reflected as a decrease in insulin AUC during an IVGTT (30). ISIS 113715 was generally well tolerated and no subjects experienced hypoglycemia, even after a 16-h fast. A pilot study was also conducted in which the effects of ISIS 113715 were studied in drug naïve subjects with type 2 diabetes. In that study, ISIS 113715 caused a reduction in both fed and fasted glucose levels as well as total and low-density lipoprotein cholesterol concentrations, which is consistent with the primate data in the present study (30). The dose that produced significant effects in drug-naïve human diabetic subjects was 200 mg/wk (~2 mg/kg, as opposed to 20 mg/kg in the current monkey study). To date, the drug has been evaluated up to doses of 600 mg/wk, and there have been no clinically significant effects on any organ system. ISIS 113715 has been well tolerated in clinical studies to date.

In summary, ISIS 113715 treatment reduces PTP-1B expression in insulin-sensitive tissues in monkeys, resulting in significant improvements of insulin sensitivity and reductions in fasting insulin concentrations in both normal-weight and obese, insulinresistant monkeys. Concentrations of adiponectin, an insulinsensitizing adipocyte-derived hormone, are low in obese monkeys and increase with ISIS 113715 treatment in proportion to the improvements of insulin sensitivity. Therefore, using PTP-1B antisense drugs to overcome defects in insulin signaling may be a promising approach for the treatment and prevention of obe-



FIG. 7. Inhibition of PTP-1B with ISIS 113715 increases circulating concentrations of total and HMW adiponectin in obese, insulin-resistant rhesus monkeys. A, Individual changes in adiponectin concentrations in low-dose/dose-escalation monkeys and high-dose-treated monkeys. B, Examination of adiponectin multimer distribution in the high-dose-treated monkeys showed a preferential increase in the HMW oligomers. C, In the entire group of monkeys whose insulin sensitivity improved as a result of the treatment (n = 19), the changes in plasma adiponectin concentrations were correlated with the improvements in insulin sensitivity, as assessed by insulin AUC in response to an IVGTT (r = -0.47, P = 0.042). Data are shown as mean \pm SEM. **, P < 0.01; ***, P < 0.0001. MMW, Medium molecular weight; LMW, low molecular weight.

sity-associated insulin resistance and type 2 diabetes, devastating diseases that are rapidly increasing in prevalence worldwide.

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References

- Kennedy BP, Ramachandran C 2000 Protein tyrosine phosphatase-1B in diabetes. Biochem Pharmacol 60:877–883
- Ahmad F, Azevedo JL, Cortright R, Dohm GL, Goldstein BJ 1997 Alterations in skeletal muscle protein-tyrosine phosphatase activity and expression in insulin-resistant human obesity and diabetes. J Clin Invest 100:449–458
- Ahmad F, Considine RV, Bauer TL, Ohannesian JP, Marco CC, Goldstein BJ 1997 Improved sensitivity to insulin in obese subjects following weight loss is accompanied by reduced protein-tyrosine phosphatases in adipose tissue. Metabolism 46:1140–1145
- Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, Normandin D, Cheng A, Himms-Hagen J, Chan CC, Ramachandran C, Gresser MJ, Tremblay ML, Kennedy BP 1999 Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science 283:1544–1548
- Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, Moghal N, Lubkin M, Kim YB, Sharpe AH, Stricker-Krongrad A, Shulman GI, Neel BG, Kahn BB 2000 Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol 20:5479–5489
- Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, Kim YB, Elmquist JK, Tartaglia LA, Kahn BB, Neel BG 2002 PTP1B regulates leptin signal transduction *in vivo*. Dev Cell 2:489–495
- 7. Egawa K, Maegawa H, Shimizu S, Morino K, Nishio Y, Bryer-Ash M, Cheung AT, Kolls JK, Kikkawa R, Kashiwagi A 2001 Protein-tyrosine phosphatase-1B negatively regulates insulin signaling in 16 myocytes and Fao hepatoma cells. J Biol Chem 276:10207–10211
- Ahmad F, Li PM, Meyerovitch J, Goldstein BJ 1995 Osmotic loading of neutralizing antibodies demonstrates a role for protein-tyrosine phosphatase 1B in negative regulation of the insulin action pathway. J Biol Chem 270:20503– 20508
- 9. Zhang S, Zhang ZY 2007 PTP1B as a drug target: recent developments in PTP1B inhibitor discovery. Drug Discov Today 12:373–381
- 10. Zinker BA, Rondinone CM, Trevillyan JM, Gum RJ, Clampit JE, Waring JF, Xie N, Wilcox D, Jacobson P, Frost L, Kroeger PE, Reilly RM, Koterski S, Opgenorth TJ, Ulrich RG, Crosby S, Butler M, Murray SF, McKay RA, Bhanot S, Monia BP, Jirousek MR 2002 PTP1B antisense oligonucleotide lowers PTP1B protein, normalizes blood glucose, and improves insulin sensitivity in diabetic mice. Proc Natl Acad Sci USA 99:11357–11362
- Waring JF, Ciurlionis R, Clampit JE, Morgan S, Gum RJ, Jolly RA, Kroeger P, Frost L, Trevillyan J, Zinker BA, Jirousek M, Ulrich RG, Rondinone CM 2003 PTP1B antisense-treated mice show regulation of genes involved in lipogenesis in liver and fat. Mol Cell Endocrinol 203:155–168
- Rondinone CM, Trevillyan JM, Clampit J, Gum RJ, Berg C, Kroeger P, Frost L, Zinker BA, Reilly R, Ulrich R, Butler M, Monia BP, Jirousek MR, Waring JF 2002 Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. Diabetes 51:2405–2411
- 13. Shimizu S, Ugi S, Maegawa H, Egawa K, Nishio Y, Yoshizaki T, Shi K, Nagai Y, Morino K, Nemoto K, Nakamura T, Bryer-Ash M, Kashiwagi A 2003 Protein-tyrosine phosphatase 1B as new activator for hepatic lipogenesis via sterol regulatory element-binding protein-1 gene expression. J Biol Chem 278: 43095–43101
- 14. Ruffolo SC, Forsell PK, Yuan X, Desmarais S, Himms-Hagen J, Cromlish W,

Wong KK, Kennedy BP 2007 Basal activation of p70S6K results in adipose-specific insulin resistance in protein-tyrosine phosphatase $1B^{-/-}$ mice. J Biol Chem 282:30423–30433

- Kadowaki T, Yamauchi T 2005 Adiponectin and adiponectin receptors. Endocr Rev 26:439–451
- Havel PJ 2004 Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. Diabetes 53(Suppl 1):S143–S151
- Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, Chen CL, Tai TY, Chuang LM 2001 Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 86:3815–3819
- Yu JG, Javorschi S, Hevener AL, Kruszynska YT, Norman RA, Sinha M, Olefsky JM 2002 The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. Diabetes 51:2968–2974
- 19. Young PW, Cawthorne MA, Coyle PJ, Holder JC, Holman GD, Kozka IJ, Kirkham DM, Lister CA, Smith SA 1995 Repeat treatment of obese mice with BRL 49653, a new potent insulin sensitizer, enhances insulin action in white adipocytes. Association with increased insulin binding and cell-surface GLUT4 as measured by photoaffinity labeling. Diabetes 44:1087–1092
- Monia BP, Lesnik EA, Gonzalez C, Lima WF, McGee D, Guinosso CJ, Kawasaki AM, Cook PD, Freier SM 1993 Evaluation of 2'-modified oligonucleotides containing 2'-deoxy gaps as antisense inhibitors of gene expression. J Biol Chem 268:14514–14522
- 21. Kratz M, Swarbrick MM, Callahan HS, Matthys CC, Havel PJ, Weigle DS 2008 Effect of dietary n-3 polyunsaturated fatty acids on plasma total and high-molecular-weight adiponectin concentrations in overweight to moderately obese men and women. Am J Clin Nutr 87:347–353
- Levin AA 1999 A review of the issues in the pharmacokinetics and toxicology of phosphorothioate antisense oligonucleotides. Biochim Biophys Acta 1489: 69–84
- 23. Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y 2001 Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 50:1126–1133
- 24. Bodkin NL, Alexander TM, Ortmeyer HK, Johnson E, Hansen BC 2003 Mortality and morbidity in laboratory-maintained Rhesus monkeys and effects of long-term dietary restriction. J Gerontol A Biol Sci Med Sci 58:212–219
- 25. Pajvani UB, Hawkins M, Combs TP, Rajala MW, Doebber T, Berger JP, Wagner JA, Wu M, Knopps A, Xiang AH, Utzschneider KM, Kahn SE, Olefsky JM, Buchanan TA, Scherer PE 2004 Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J Biol Chem 279:12152–12162
- Geary RS, Watanabe TA, Truong L, Freier S, Lesnik EA, Sioufi NB, Sasmor H, Manoharan M, Levin AA 2001 Pharmacokinetic properties of 2'-O-(2methoxyethyl)-modified oligonucleotide analogs in rats. J Pharmacol Exp Ther 296:890–897
- Haj FG, Zabolotny JM, Kim YB, Kahn BB, Neel BG 2005 Liver-specific protein-tyrosine phosphatase 1B (PTP1B) re-expression alters glucose homeostasis of PTP1B^{-/-}mice. J Biol Chem 280:15038–15046
- Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR 2000 Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 6:87–97
- McKay RA, Murray SF, Booten S, Bhanot S, Monia BP 2003 Effects of a Novel PTP-1B antisense oligonucleotide Inhibitor (ISIS 113715) on PTP-1B expression in different liver cell types. Diabetes 52(Suppl 1):A142
- Bhanot S 2008 Developing antisense drugs for metabolic diseases: a novel therapeutic approach. In: Crooke ST, ed. Antisense drug technology: principles, strategies, and applications. 2nd ed. New York: Marcel Dekker, Inc. 641–663

- 31. Winter CL, Lange JS, Davis MG, Gerwe GS, Downs TR, Peters KG, Kasibhatla B 2005 A nonspecific phosphotyrosine phosphatase inhibitor, bis(maltolato-)oxovanadium(IV), improves glucose tolerance and prevents diabetes in Zucker diabetic fatty rats. Exp Biol Med (Maywood) 230:207–216
- 32. Swarbrick MM, Austrheim-Smith IT, Stanhope KL, Van Loan MD, Ali MR, Wolfe BM, Havel PJ 2006 Circulating concentrations of high-molecularweight adiponectin are increased following Roux-en-Y gastric bypass surgery. Diabetologia 49:2552–2558
- Askew EW, Huston RL, Plopper CG, Hecker AL 1975 Adipose tissue cellularity and lipolysis. Response to exercise and cortisol treatment. J Clin Invest 56:521–529
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante Jr AW 2003 Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112:1796–1808
- 35. Bahceci M, Gokalp D, Bahceci S, Tuzcu A, Atmaca S, Arikan S 2007 The correlation between adiposity and adiponectin, tumor necrosis factor α, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults? J Endocrinol Invest 30:210–214
- Shadoan MK, Kavanagh K, Zhang L, Anthony MS, Wagner JD 2007 Addition of medroxyprogesterone acetate to conjugated equine estrogens results in insulin resistance in adipose tissue. Metabolism 56:830–837
- Imbeault P 2007 Environmental influences on adiponectin levels in humans. Appl Physiol Nutr Metab 32:505–511
- Swarbrick MM, Havel PJ 2008 Physiological, pharmacological, and nutritional regulation of circulating adiponectin concentrations in humans. Metab Syndr Relat Disord 6:87–102
- 39. Kemnitz JW, Elson DF, Roecker EB, Baum ST, Bergman RN, Meglasson MD 1994 Pioglitazone increases insulin sensitivity, reduces blood glucose, insulin, and lipid levels, and lowers blood pressure, in obese, insulin-resistant rhesus monkeys Diabetes 43:204–211
- Seo JB, Moon HM, Noh MJ, Lee YS, Jeong HW, Yoo EJ, Kim WS, Park J, Youn BS, Kim JW, Park SD, Kim JB 2004 Adipocyte determination- and differentiation-dependent factor 1/sterol regulatory element-binding protein 1c regulates mouse adiponectin expression. J Biol Chem 279:22108–22117
- 41. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, Ouchi N, Kuriyama H, Hotta K, Nakamura T, Shimomura I, Matsuzawa Y 2001 PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 50:2094–2099
- 42. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, Nelson M, Ong E, Olefsky JM, Evans RM 2003 Adipose-specific peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle. Proc Natl Acad Sci USA 100:15712–15717
- Bartness TJ, Song CK 2007 Brain-adipose tissue neural crosstalk. Physiol Behav 91:343–351
- 44. Cnop M, Havel PJ, Utzschneider KM, Carr DB, Sinha MK, Boyko EJ, Retzlaff BM, Knopp RH, Brunzell JD, Kahn SE 2003 Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 46:459–469
- 45. Nicklin PL, Bayley D, Giddings J, Craig SJ, Cummins LL, Hastewell JG, Phillips JA 1998 Pulmonary bioavailability of a phosphorothioate oligonucleotide (CGP 64128A): comparison with other delivery routes. Pharm Res 15:583–591
- van Houten M, Posner BI, Kopriwa BM, Brawer JR 1980 Insulin binding sites localized to nerve terminals in rat median eminence and arcuate nucleus. Science 207:1081–1083
- Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, Kahn BB 2006 Neuronal PTP1B regulates body weight, adiposity and leptin action. Nat Med 12:917–924