Chronic treatment with growth hormone stimulates adiponectin gene expression in 3T3-L1 adipocytes

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Abstract **Growth hormone (GH) is an important regulator of adiposity and systemic energy metabolism. Here, we have investigated the effects of GH on production of adiponectin, an anti-diabetic and anti-atherogenic hormone secreted exclusively from adipocytes. Analysis using real time quantitative PCR revealed that GH significantly increased adiponectin gene expression in a dose-dependent manner. Time course study showed that the expression of adiponectin gene started to increase only after 30 h of GH treatment (10^{-8} M), suggesting it to be a chronic effect. GH-mediated induction of adiponectin gene expression was completely blocked by treatment with the Janus kinase2 (JAK2) inhibitor AG490 and the P38 mitogen activated protein (MAP) kinase inhibitor SB203580, while the specific inhibitors of phosphatidylinositol-3-kinase (LY294002) and p70S6 kinase (rapamycin) moderately enhanced GHs effect. Co-incubation of adipocytes with GH and the PPARγ agonist rosiglitazone produced additive effects on induction of adiponectin gene expression. These results collectively suggest that GH increases adiponectin gene expression through the JAK2-P38 MAP kinase pathway, and that elevation of adiponectin production might represent a novel mechanism by which GH regulates systemic energy metabolism and insulin sensitivity.**

Keywords: Adiponectin; Adipocyte; Growth hormone; Insulin sensitivity; Adipokine

1. Introduction

Adipose tissue is now recognized to be an important endocrine organ, secreting a variety of polypetides (adipokines) that are involved in the regulation of energy metabolism, immune response and cardiovascular tone [1,2]. Adiponectin (also called ACRP30, adipoQ or Apm1) is an important adipokine exclusively secreted from adipose tissue [3]. This protein belongs to the soluble defense collagen superfamily that contains a modular design comprising an NH_2-terminal collagen-repeat domain and a COOH-terminal, characteristic complement factor C1q-like globular head domain. Growing evidence suggests that adiponectin is an insulin-sensitizing hormone with direct anti-diabetic, anti-atherogenic and anti-inflammatory potentials [2,3].

It has been demonstrated that both central and peripheral administration of recombinant adiponectin cause sustained weight loss in mice without reducing food intake [4,5]. Injection of recombinant full-length adiponectin produced from mammalian cells abolishes hyperglycemia in several diabetic animal models, possibly through downregulation of the hepatic expression of the gluconeogenic enzymes [6]. Chronic treatment with adiponectin could restore insulin sensitivity in both high fat (HF) fed and lipoatropic mice, by increasing β-oxidation of fatty acid in muscle, and thus decreasing muscular triglyceride content [7]. Furthermore, adenovirus-mediated expression of adiponectin alleviates atherosclerosis in apoE deficient mice. We have recently found that chronic administration of recombinant full-length adiponectin generated from mammalian cells can alleviate alcoholic and non-alcoholic fatty liver diseases associated with obese ob/ob mice, and can also ameliorate dyslipidemia and insulin resistance in these animal models [8]. In vitro studies have shown that recombinant adiponectin can prevent foam cell formation [9], inhibit the proliferation of smooth muscle cells seen when they are stimulated by various growth factors [10], and protect pancreatic islet β cells from apoptosis [11]. Two more recent independent knockout studies showed that depletion of adiponectin expression in mice causes moderate insulin resistance, glucose intolerance and increased neointimal formation following mechanical injury [12,13], suggesting that adiponectin deficiency is one of the major contributors to the causation of these metabolic disorders.

Plasma levels of adiponectin correlate closely with systemic insulin sensitivity, and decreased adiponectin concentrations (hypoadiponectinemia) were observed in patients with type 2 diabetes, insulin resistance or coronary heart disease [14–16]. Several prospective studies suggest that a decline in plasma adiponectin concentration preceded the decrease in insulin sensitivity, suggesting that adiponectin deficiency might be an important causative factor of insulin resistance [17,18]. Indeed, several insulin resistance inducing factors, such as tumor necrosis factor α, interleukin 6, dexmethasone and isoproterenol, have been shown to reduce adiponectin production [19–21]. On the other hand, the PPARγ agonists thiazolidinediones (TZD), which are used clinically as insulin sensitizing drugs, could increase adiponectin expression and its plasma concentrations in rodents and human subjects [22,23].

Growth hormone (GH) is an important regulator of systemic energy metabolism and insulin sensitivity [24]. Adipose tissue is one of the major targets of GH, where both full-length and the truncated GH receptors are expressed [25]. Opposing

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effects of GH on glucose and lipid metabolism have been reported in adipocytes: acute insulin-like effects and chronic insulin-antagonizing effects [24]. In the present study, we investigated the direct effect of GH on adiponectin production from adipocytes. Our results revealed that chronic treatment with GH could increase adiponectin production in a time- and dose-dependent manner. GH-induced adiponectin expression was completely blocked by the Janus kinase2 (JAK2) specific inhibitor AG490 and the P38 mitogen activated protein (MAP) kinase inhibitor SB203580. Furthermore, we observed that insulin inhibited the action of GH on adiponectin gene expression.

2. Materials and methods

2.1. Materials

Insulin, dexamethasone, methyl-isobutyl-xanthine, PD98059, SB203580 and LY294002 were purchased from Sigma (Saint Louis, MO, USA). Genestein and AG490 are products of Calbiochem (La Jolla, CA, USA). Human recombinant GH and rosiglitazone were obtained from Lilly France (S.A. Fersheim, France) and Alexis Biochemicals (Lausanne, Switzerland), respectively. Trizol Reagent, Superscript first-strand cDNA synthesis system and lipofectamine 2000 were purchased form Invitrogen (Carlsbad, CA, USA). Dual luciferase reporter system and the reporter vectors pGL3-basic and pRL-CMV are the products of Promega Corporation (Madison, USA).

2.2. Cell culture and differentiation

3T3-L1 cells were maintained as subconfluent cultures in Dubecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Adipocyte differentiation was induced as we previously described [26].

2.3. Analysis of adiponectin gene expression by Northern blot and real time quantitative RT-PCR

Total RNA was extracted from 3T3-L1 adipocytes using Trizol Reagents according to the manufacturer's instructions. 1 μg of total RNA was transcribed into cDNA with the Superscript first-strand cDNA synthesis system. Taqman real-time quantitative PCR was conducted according to the manufacturer's instructions (Applied Biosystems, Foster, CA, USA). Northern blot analysis of the adiponectin gene abundance was performed as we previously described [27].

2.4. Quantification of adiponectin concentration by ELISA

The conditioned culture medium from 3T3-L1 adipocytes treated without or with GH (10^{-8} M) was collected at different time points, diluted with PBS containing 1% BSA, and then quantified using a sandwich ELISA method as we previously described [27].

2.5. Cloning of mouse adiponectin promoter and construction of the luciferase reporter vector

Mouse genomic DNA extracted from 3T3-L1 adipocytes was used as template for PCR amplification. A 1.0 kb promoter segment corresponding to −1037 to +19 bp of the mouse adiponectin gene was amplified using the forward primer CGATTGCTAGCCCTTTTAGGAGCAGTTTAGTGAGTG and the reverse primer AGTCACTCGAGGTCAGATCCACTGACAATCGTACAG, respectively. The DNA fragment was digested with *Nhe*I and *Xho*I, and subcloned into PGL3-basic vector. The sequence of the cloned DNA fragment was confirmed by DNA sequencing.

2.6. Transient transfection and luciferase reporter assays

3T3-L1 adipocytes at day 7 after differentiation were transfected with the luciferase reporter vectors using Lipofectamine 2000. Luciferase assays were conducted using the Dual-Luciferase Reporter system (Promega). Transiently transfected cells were solubilized in 100 μl of lysis buffer. After centrifugation to remove cell debris, 20 μl of the cell lysate was used to measure luciferase activity according to the manufacturer's instructions. In each measurement, luciferase units were normalized for background and for transfection efficiency as determined by the '*Renilla*' luciferase activity of the co-transfected pRL-CMV plasmid.

2.7. Statistical analysis

Results were reproduced in at least four independent experiments. The results are presented as means of at least triplicate determinations ±S.D. Significance was determined by Student's t test or one-way ANOVA. In all statistical comparisons, a P value of less than 0.05 was considered statistically significant.

3. Results

3.1. GH induces expression of adiponectin gene in a time- and dose-dependent manner in 3T3-L1 adipocytes

To evaluate the effect of GH on adiponectin gene expression, we treated 3T3-L1 adipocytes with different concentrations of GH for 40 h. Real time quantitative PCR analysis revealed that several dosages of GH (from 10^{-11} to 10^{-7} M) significantly increased adiponectin gene expression, while supra-physiological concentration of GH (10^{-6} M) had no obvious effect (Fig. 1A). This result was further confirmed by Northern blot analysis (Fig. 1B). GH treatment under exactly the same condition did not influence the mRNA expression of aP2 and PPARγ, the two well-established differentiation markers of adipocytes, suggesting that GH-induced adiponectin gene expression is not due to its effect on adipogenesis (Fig. 1C).

A time course study revealed that a significant induction of adiponectin gene expression was detected only after 30 h of GH treatment, and reached a maximum after 40 h (Fig. 2A), suggesting GH action on adiponection expression to be of a chronic nature. Quantitative ELISA analysis demonstrated that adiponectin concentrations in the conditioned medium were significantly increased only at 40 h after GH treatment (Fig. 2B). The maximal elevation was detected at 60 h after GH treatment. This result suggests that the increase of adiponectin secretion follows behind the GH-induced elevation of adiponectin gene expression in 3T3-L1 adipocytes.

3.2. GH-induced expression of adiponectin gene is blocked by inhibition of the JAK2-P38 MAP kinase pathway

GH initiates its multiple biological effects by inducing dimerization of its receptor followed by activation of JAK2. Activated JAK2 is responsible for tyrosine phosphorylation of the GH receptors and subsequent activation of several kinases, including phosphatidylinositol-3 (PI-3)-kinase, P44/42 MAP kinase and P38 MAP kinase [25]. p70S6 kinase is an important signaling protein downstream of PI-3-kinase implicated in the regulation of protein synthesis [28]. We next investigated the role of these signaling pathways on GH-induced adiponectin gene expression, by using specific inhibitors of the aforementioned kinases. GH-mediated induction of adiponectin gene expression was completely blocked by either the JAK2 inhibitor AG490, the tyrosine kinase inhibitor genestein or the P38 MAP kinase inhibitor SB203586 (Fig. 3). The P42/44 MAP kinase inhibitor PD98059 had no obvious effect. On the other hand, treatment with the PI-3-kinase inhibitor LY294002 and the p70S6 kinase inhibitor rapamycin enhanced the action of GH on induction of the adiponectin gene expression, suggesting that PI-3-kinase/p70S6 kinase pathway is a suppressor of adiponectin gene expression.

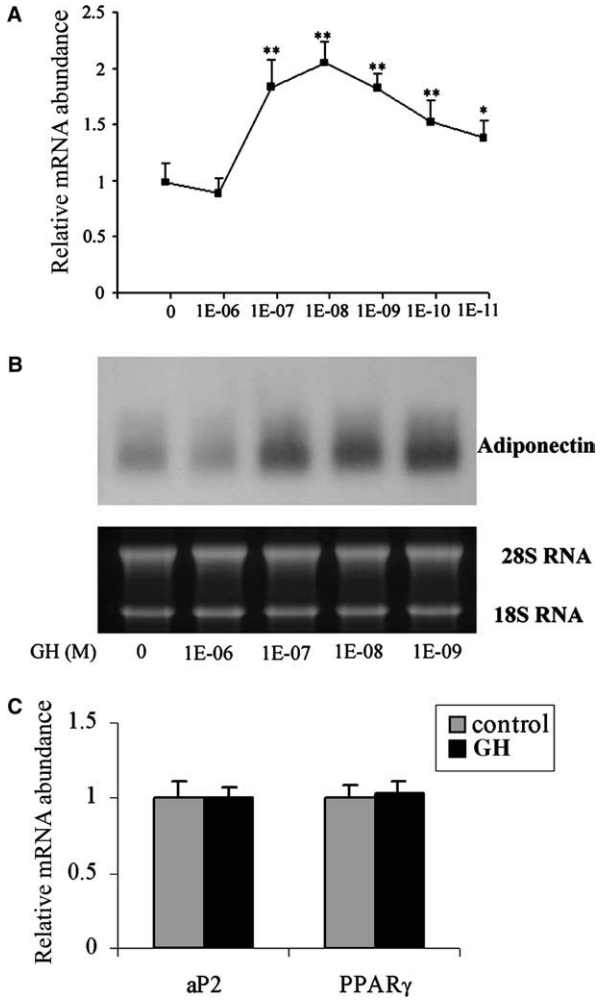

Fig. 1. Effects of GH on expression of the adiponectin, aP2 and PPARγ genes in 3T3-L1 adipocytes. In panel A, 3T3-L1 adipocytes (day 7 after differentiation) were untreated or treated with different concentrations of GH for 40 h. Total RNA purified from these samples was subjected to quantitative PCR analysis to determine the levels of adiponectin gene expression as described in Section 2.3. The steady state abundance of adiponectin RNA is expressed relative to untreated control cells. *, $P < 0.05$ and **, $P < 0.01$ versus untreated samples ($n = 6$). Panel B is a representative autoradiogram from Northern blot analysis of adiponectin RNA ($n = 5$). Panel C, the steady state abundance of aP2 and PPARγ RNA from 3T3-L1 adipocytes treated without or with GH (10^{-8}) for 40 h was analyzed by quantitative PCR as in panel A ($n = 5$).

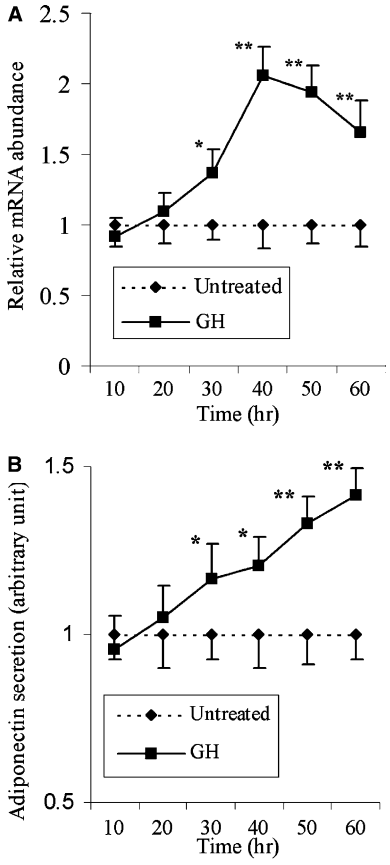

Fig. 2. Time-dependent induction of adiponectin gene expression and protein secretion by GH. (A) 3T3-L1 adipocytes were treated without or with 10^{-8} M GH for the indicated periods of time. The relative abundance of adiponectin RNA was determined by quantitative PCR. (B) The cells were treated as in panel A. An aliquot of the conditioned medium was collected at each time point and then subjected to ELISA analysis of adiponectin concentration, as we previously described [27]. For comparison, the levels of the adiponectin RNA (panel A) and protein concentration (panel B) from each time point of the untreated samples were arbitrarily set at 1. *, $P < 0.05$ and **, $P < 0.01$ versus untreated samples in each time point ($n = 4$–6).

3.3. GH and PPARγ induce adiponectin gene expression via distinct pathways

The expression of adiponectin has been shown to be under the control of the transcription factor PPARγ [29]. The PPARγ agonists TZD can increase plasma adiponectin levels in humans and mice [22]. Consistent with previous reports, we found that the PPARγ agonist rosiglitazone enhanced adiponectin mRNA expression by ~1.8-fold (Fig. 4A), while GH increased adiponectin gene expression by ~2-fold. Co-incubation of cells with GH and rosiglitazone elevated the steady state mRNA abundance of adiponectin gene by ~4-fold, suggesting that the effects of GH and rosiglizatone on adiponectin gene expression are additive. Furthermore, treatment of adipocytes with rosiglitazone enhanced the activity of the −1.0 kb mouse adiponectin gene promoter by ~2.8-fold, whereas GH had no significant effect. These results suggest that GH and the PPARγ agonist induce adiponectin gene expression through distinct mechanisms.

3.4. GH and insulin exert opposite effects on adiponectin gene expression in 3T3-L1 adipocytes

Insulin has been shown to be a negative regulator of adiponectin gene expression [20,30]. Insulin and GH exert many opposite effects on adipocytes, including lipolysis, lipogenesis and glucose uptake [24]. Here, we investigated the interactions between GH and insulin on adiponectin gene expression. Consistent with previous reports, treatment of adipocytes with 10 nM of insulin alone significantly decreased expression level of the adiponectin gene (Fig. 5). In addition, GH-induced adiponectin gene expression was completely blocked by insulin treatment.

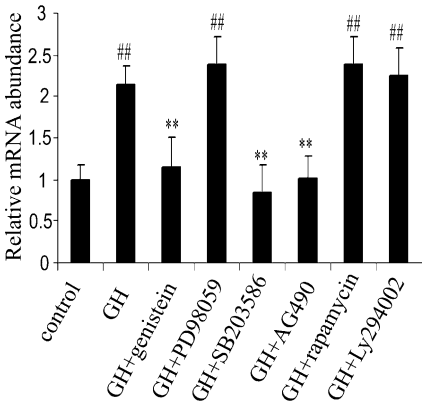

Fig. 3. Effects of different pharmacological inhibitors on GH-mediated induction of adiponectin gene expression. 3T3-L1 adipocytes were untreated (control), or treated with GH, or GH plus 25 μM genestein, 25 μM PD98059, 25 μM SB203586, 25 μM AG490, 1 μM rapamycin or 10 μM LY294002. 40 h after the treatment, 1 μg of total RNA from each sample was subjected to quantitative PCR analysis of adiponectin RNA. **, $P < 0.01$ versus control; ##, $P < 0.05$ and #, $P < 0.01$ versus GH treated samples ($n = 6$–8). Note that none of these pharmacological inhibitors significantly affect the basal mRNA expression of the adiponectin gene (data not shown).

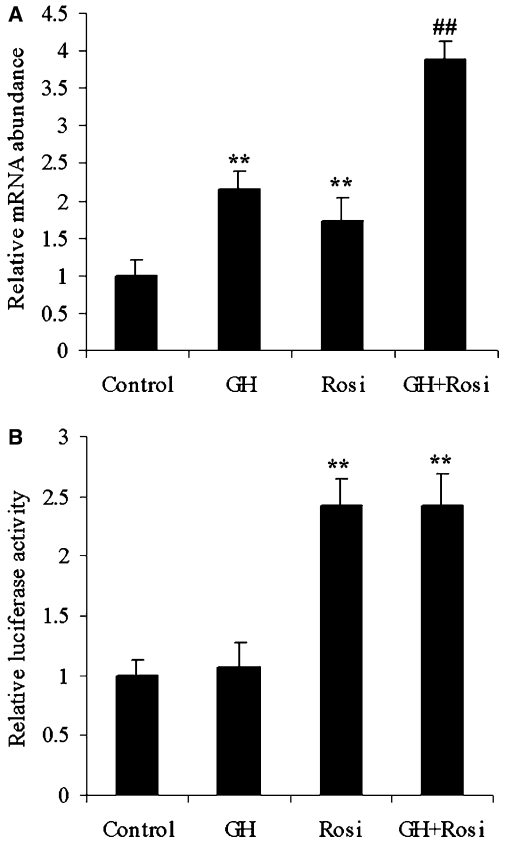

Fig. 4. GH and rosiglitazone augmented adiponectin gene expression through different pathways. In panel A, 3T3-L1 adipocytes were untreated (control), or treated with 10 μM rosiglitazone (Rosi), 10^{-8} M GH, or 10 μM rosiglitazone plus 10^{-8} M GH for 40 h. Adiponectin RNA was quantified as described in Fig. 1. *, $P < 0.01$ versus control; ##, $P < 0.01$ versus samples treated with GH or Rosi alone ($n = 4$–6). In panel B, 3T3-L1 adipocytes grown in a 12-well dish were transfected with the luciferase reporter vector driven by the 1.0 kb adiponectin promoter plus PRL-CMV plasmid. 4 h after transfection, the cells were treated as in panel A. Firefly and *Renilla* luciferase reporter activity was measured as described in Section 2.5. The adiponectin promoter activity was normalized by pRL-CMV Renilla luciferase activity. *, $P < 0.01$ versus control ($n = 4$).

4. Discussions

GH is now recognized to be an important regulator of systemic metabolism and glucose homeostasis. Although several studies have demonstrated that exogenous administration of supra-physiological doses of GH results in diabetogenic effects [31], it has also been demonstrated that GH can decrease hyperglycemia and improve insulin sensitivity [32,33]. GH supplement therapy in both obese individuals and GH deficient subjects has shown various beneficial effects, especially in decreasing body fat percentage and in improving lipid metabolism, though the long-term outcome of this treatment remains to be established.

In addition to its role in regulating glucose and lipid metabolism [25], GH has been shown to modulate the production of adipocyte-derived adipokines, such as leptin and resistin [34,35]. In the present study, we demonstrated that chronic treatment of 3T3-L1 adipocytes with physiological concentrations of GH led to a dose-dependent induction of adiponectin gene expression (Fig. 1). This induction was time-dependent, being observed only after 30 h of GH treatment, reaching a maximum after 40 h (Fig. 2). In keeping with these findings, no effect of GH on adiponectin mRNA levels was observed in a previous study in which 3T3-L1 cells were treated with GH for 16 h [20]. Our findings suggest that an induction of adiponectin gene expression could constitute one mechanism, whereby treatment with physiological doses of GH leads to improved insulin sensitivity in GH deficient insulin resistant adults [33]. Indeed, in a recent study, a rise in serum adiponectin was observed following GH replacement in women with GH deficiency [35].

Although the net outcome of GH stimulation at several dosages is to increase steady-state mRNA abundance of the adiponectin gene, several signaling pathways evoked by this hormone appear to play opposite roles. Treatment with the P38 MAP kinase inhibitor SB203580 led to the complete inhibition of GH-induced adiponectin gene expression, suggesting that this kinase is a positive regulator of adiponectin gene expression. On the other hand, GH-induced elevation of adiponectin gene expression was further enhanced by treatment with the inhibitors of PI-3-kinase or p70S6 kinase, indicating that activation of PI-3-kinase/p70S6 kinase pathway suppresses the expression of this gene. The opposing actions of these two GH-induced pathways could explain why supra-physiological concentration of GH (10^{-6}) had no obvious effect on adiponectin gene expression (Fig. 1). Given the existence of both full-length and truncated forms of GH receptors with different affinities in adipocytes [36], it is possible that different concentrations of GH can differentially activate the aforementioned kinase pathways. Upon treatment with a supra-physiological concentration of GH (10^{-6}), the P38 MAP kinase pathway-mediated positive regulation of adiponectin gene expression might be completely counteracted by the negative effect of the PI-3-kinase/p70S6 pathway.

Our finding that insulin can inhibit the GH-induced elevation of the adiponectin gene expression further supports the negative role of PI-3-kinase/p70S6 kinase pathway in this process (Fig. 5). Several recent studies demonstrated that in-

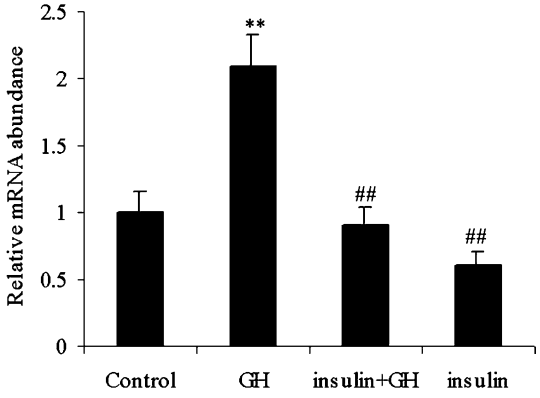

Fig. 5. GH-induced adiponectin gene expression is suppressed by insulin. 3T3-L1 adipocytes were untreated, or treated with 10^{-8} GH, 10 nM insulin plus 10^{-8} GH, or 10 nM insulin alone. 40 h after treatment, the steady state abundance of adiponectin RNA was quantified as in Fig. 1. **, $P < 0.01$ versus control; ##, $P < 0.01$ versus GH treated group ($n = 4$–6).

sulin is the negative regulator of adiponectin production in vitro as well as in vivo [20,30]. Clinical data revealed that there is an inverse relationship between plasma levels of insulin and adiponectin [16]. Furthermore, infusion of insulin into healthy individuals can cause a significant reduction of plasma adiponectin. The role of insulin as a negative regulator of adiponectin production was further confirmed by the finding that adipose tissue-specific ablation of the insulin receptor resulted in increased plasma adiponectin levels and systemic insulin sensitivity [37]. It has been proposed that hyperinsulinemia is at least partly responsible for hypoadiponectinemia during the dynamic phase of insulin resistance. In line with our finding, a previous study has found that inhibition of either PI-3-kinase or p70S6 using their specific pharmacological inhibitors partially reversed insulin-mediated suppression of adiponectin gene expression [20]. Co-incubation of GH with insulin will therefore lead to further activation of the PI-3-kinase/p70S6 kinase pathway, which will in turn counteract the positive regulation of adiponectin by other pathways.

The transcriptional events that underlie the adiponectin gene expression remain poorly understood. The transcription factor PPARγ, a key regulator of adipogenesis, has been shown to transactivate adiponectin gene expression [23]. The PPARγ agonist rosiglitazone could increase adiponectin production in both animals and patients with diabetes [22]. A more recent study has mapped the PPARγ-responsive elements to a DNA fragment between −285 and −273 of the human adiponectin gene [29]. Nevertheless, our current study suggests that PPARγ is not involved in GH-induced gene expression. This notion is supported by the fact that co-incubation of adipocytes with rosiglitazone and GH had additive effect on induction of adiponectin gene expression and that the activity of a 1.0 kb adiponectin gene promoter was increased by rosiglitazone, but not by GH (Fig. 4). Given that GHs action on adiponectin is chronic (Fig. 2) and that it has no effect on the activity of the −1.0 kb mouse adiponectin gene promoter, it is possible that this hormone might increase steady state mRNA abundance of adiponectin gene by enhancing its stability and preventing its degradation. In line with this speculation, regulation of mRNA stability has been proposed to be an important mechanism by which the expression of the adiponectin gene is modulated [38]. Nevertheless, our results do not exclude the possibility that GH transactivates adiponectin gene expression through a *cis*-DNA fragment that is outside the −1.0 kb adiponectin gene promoter region. These possibilities are currently under investigation in our laboratory.

In summary, we demonstrate for the first time that chronic treatment of GH significantly enhances expression of the anti-diabetic and anti-atherogenic hormone adiponectin. Interestingly, a recent report found that GH is also the positive regulator of the newly identified adiponectin receptors [39], suggesting that regulation of the adiponectin pathway might represent an important aspect of GH actions. Many recent studies have shown that adiponectin replacement therapy might represent a novel strategy for the treatment of a variety of diseases, including insulin resistance, type 2 diabetes, atherosclerosis and liver diseases [5–8]. However, the adiponectin protein based-therapy is limited by the fact that the circulating concentration of adiponectin (5–30 μg/ml) is very high [14], which would require large quantity of the recombinant protein. Further investigation of the signaling pathways that underlie GH-induced adiponectin gene expression might help to design novel therapeutic strategies that act through increasing endogenous adiponectin production.

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References

[1] Lyon, C.J., Law, R.E., Hsueh, W.A., Chaldakov, G.N., Stankulov, I.S., Hristova, M. and Ghenev, P.I. (2003) Endocrinology 144, 2195–2200.
[2] Chaldakov, G.N., Stankulov, I.S., Hristova, M., Ghenev, P.I., Diez, J.J. and Iglesias, P. (2003) Curr. Pharm. Des. 9, 1023–1031.
[3] Berg, A.H., Combs, T.P. and Scherer, P.E. (2002) Trends Endocrinol. Metab. 13, 84–89.
[4] Qi, Y., Takahashi, N., Hileman, S.M., Patel, H.R., Berg, A.H., Pajvani, U.B., Scherer, P.E. and Ahima, R.S. (2004) Nat. Med. 11, 11.
[5] Fruebis, J., Tsao, T.S., Javorschi, S., Ebbets-Reed, D., Erickson, M.R., Yen, F.T., Bihain, B.E. and Lodish, H.F. (2001) Proc. Natl. Acad. Sci. USA 98, 2005–2010.
[6] Berg, A.H., Combs, T.P., Du, X., Brownlee, M. and Scherer, P.E. (2001) Nat. Med. 7, 947–953.
[7] Yamauchi, T. et al. (2001) Nat. Med. 7, 941–946.
[8] Xu, A., Wang, Y., Keshaw, H., Xu, L.Y., Lam, K.S. and Cooper, G.J. (2003) J. Clin. Invest. 112, 91–100.
[9] Ouchi, N. et al. (2001) Circulation 103, 1057–1063.
[10] Arita, Y. et al. (2002) Circulation 105, 2893–2898.
[11] Rakatzi, I., Mueller, H., Ritzeler, O., Tennagels, N. and Eckel, J. (2004) Diabetologia 47, 249–258, Epub 2004 January 13.
[12] Maeda, N. et al. (2002) Nat. Med. 8, 731–737.
[13] Kubota, N. et al. (2002) J. Biol. Chem. 277, 25863–25866.
[14] Arita, Y. et al. (1999) Biochem. Biophys. Res. Commun. 257, 79–83.
[15] Hotta, K. et al. (2000) Arterioscler. Thromb. Vasc. Biol. 20, 1595–1599.
[16] Weyer, C., Funahashi, T., Tanaka, S., Hotta, K., Matsuzawa, Y., Pratley, R.E. and Tataranni, P.A. (2001) J. Clin. Endocrinol. Metab. 86, 1930–1935.
[17] Lindsay, R.S., Funahashi, T., Hanson, R.L., Matsuzawa, Y., Tanaka, S., Tataranni, P.A., Knowler, W.C. and Krakoff, J. (2002) Lancet 360, 57–58.
[18] Hotta, K., Funahashi, T., Bodkin, N.L., Ortmeyer, H.K., Arita, Y., Hansen, B.C. and Matsuzawa, Y. (2001) Diabetes 50, 1126–1133.

[19] Fasshauer, M., Klein, J., Neumann, S., Eszlinger, M. and Paschke, R. (2001) FEBS Lett. 507, 142–146.
[20] Fasshauer, M., Klein, J., Neumann, S., Eszlinger, M. and Paschke, R. (2002) Biochem. Biophys. Res. Commun. 290, 1084–1089.
[21] Fasshauer, M., Kralisch, S., Klier, M., Lossner, U., Bluher, M., Klein, J. and Paschke, R. (2003) Biochem. Biophys. Res. Commun. 301, 1045–1050.
[22] Combs, T.P. et al. (2002) Endocrinology 143, 998–1007.
[23] Maeda, N. et al. (2001) Diabetes 50, 2094–2099.
[24] Nam, S.Y. and Marcus, C. (2000) Horm. Res. 53, 87–97.
[25] Nam, S.Y., Lobie, P.E. and Marcus, C. (2000) Obes. Rev. 1, 73–86.
[26] Wang, Y., Xu, A., Knight, C., Xu, L.Y. and Cooper, G.J. (2002) J. Biol. Chem. 277, 19521–19529.
[27] Xu, A., Yin, S., Wong, L., Chan, K.W. and Lam, K.S. (2004) Endocrinology 145, 487–494.
[28] Virkamaki, A., Ueki, K. and Kahn, C.R. (1999) J. Clin. Invest. 103, 931–943.
[29] Iwaki, M., Matsuda, M., Maeda, N., Funahashi, T., Matsuzawa, Y., Makishima, M. and Shimomura, I. (2003) Diabetes 52, 1655–1663.
[30] Mohlig, M., Wegewitz, U., Osterhoff, M., Isken, F., Ristow, M., Pfeiffer, A.F. and Spranger, J. (2002) Horm. Metab. Res. 34, 655–658.
[31] Fowelin, J., Attvall, S., Lager, I. and Bengtsson, B.A. (1993) Metabolism 42, 1443–1447.
[32] Yuen, K. et al. (2002) J. Clin. Endocrinol. Metab. 87, 1989–1995.
[33] Hwu, C.M. et al. (1997) J. Clin. Endocrinol. Metab. 82, 3285–3292.
[34] Delhanty, P.J., Mesotten, D., McDougall, F. and Baxter, R.C. (2002) Endocrinology 143, 2445–2448.
[35] Eden Engstrom, B., Burman, P., Holdstock, C. and Karlsson, F.A. (2003) J. Clin. Endocrinol. Metab. 88, 5193–5198.
[36] Iida, K., Takahashi, Y., Kaji, H., Yoshioka, S., Murata, M., Iguchi, G., Okimura, Y. and Chihara, K. (2003) Mol. Cell. Endocrinol. 210, 21–29.
[37] Bluher, M., Michael, M.D., Peroni, O.D., Ueki, K., Carter, N., Kahn, B.B. and Kahn, C.R. (2002) Dev. Cell. 3, 25–38.
[38] Halleux, C.M., Takahashi, M., Delporte, M.L., Detry, R., Funahashi, T., Matsuzawa, Y. and Brichard, S.M. (2001) Biochem. Biophys. Res. Commun. 288, 1102–1107.
[39] Fasshauer, M., Klein, J., Kralisch, S., Klier, M., Lossner, U., Bluher, M. and Paschke, R. (2004) FEBS Lett. 558, 27–32.