Biochemical and Biophysical Research Communications 376 (2008) 21–25

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Inhibition of GIP signaling modulates adiponectin levels under high-fat diet in mice

Rei Naitoh [a], Kazumasa Miyawaki [a], Norio Harada [a], Wataru Mizunoya [b], Kentaro Toyoda [a], Tohru Fushiki [b], Yuichiro Yamada [a,c], Yutaka Seino [a,d], Nobuya Inagaki [a,e,*]

[a] Department of Diabetes and Clinical Nutrition, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan
[b] Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan
[c] Department of Endocrinology and Diabetes and Geriatric Medicine, Akita University School of Medicine, Akita, Japan
[d] Kansai Electric Power Hospital, Osaka, Japan
[e] CREST of Japan Science and Technology Cooperation (JST), Kyoto, Japan

ARTICLE INFO

Article history:
Received 8 August 2008
Available online 22 August 2008

Keywords:
GIP
Fat oxidation
Adiponectin
PPAR

ABSTRACT

Gastric inhibitory polypeptide (GIP) is an incretin and directly promotes fat accumulation in adipocytes. Inhibition of GIP signaling prevents onset of obesity and increases fat oxidation in peripheral tissues under high-fat diet (HFD), but the mechanism is unknown. In the present study, we investigated the effects of inhibition of GIP signaling on adiponectin levels after 3 weeks of HFD by comparing wild-type (WT) mice and GIP receptor-deficient ($Gipr^{-/-}$) mice. In HFD-fed $Gipr^{-/-}$ mice, fat oxidation was significantly increased and adiponectin mRNA levels in white adipose tissue and plasma adiponectin levels were significantly increased compared to those in HFD-fed WT mice. In addition, the PPARα mRNA level was increased and the ACC mRNA level was decreased in skeletal muscle of HFD-fed $Gipr^{-/-}$ mice compared with those in HFD-fed WT mice. These results indicate that inhibition of GIP signaling increases adiponectin levels, resulting in increased fat oxidation in peripheral tissues under HFD.

Gastric inhibitory polypeptide (GIP) is a major incretin that potentiates insulin secretion in pancreatic β-cells in the presence of glucose [1,2]. GIP is released from duodenal endocrine K-cells after meal ingestion, and acts by binding the GIP receptor through increased intracellular cAMP [3]. The GIP receptor is expressed in pancreas, stomach, small intestine, heart, adrenal cortex, brain, lung, bone, vascular endothelium, and adipose tissue [4]. In addition to the insulinotropic effects on pancreatic β-cells, GIP is an obesity-promoting factor that directly leads to the accumulation of fat in adipocytes. In vitro studies show that GIP stimulates synthesis and secretion of lipoprotein lipase (LPL) in cultured preadiocytes [5], and that GIP promotes LPL activity in fat tissue [6]. It also has been shown that GIP stimulates glucose transport and increases fatty-acid synthesis in fat tissue [7]. Studies of GIP receptor-deficient mice ($Gipr^{-/-}$ mice) show that GIP also is an important factor in the promotion of obesity in vivo [8]. High-fat diet (HFD)-fed wild-type (WT) mice exhibit body weight gain and markedly increased visceral and subcutaneous fat mass and liver steatosis. By contrast, $Gipr^{-/-}$ mice fed HFD exhibit neither weight gain nor adiposity. Furthermore, measurement of the respiratory quotient reveals that fat is used as the preferred energy substrate in $Gipr^{-/-}$ mice. In addition, a study using IRS-$1^{-/-}$/$Gipr^{-/-}$ double-deficient mice demonstrated that inhibition of GIP signaling increases fatty-acid oxidation in peripheral tissues under diminished insulin signaling [9]. Thus, GIP plays a critical role in adiposity, but the mechanism of fat oxidation in peripheral tissues in the absence of GIP signaling is unclear.

Adiponectin is one of the major adipokines secreted from adipocytes, and stimulates fat oxidation in peripheral tissues. Adiponectin promotes AMPK activation and PPARα expression and stimulates fat oxidation in skeletal muscle and liver [10]. In the present study, we investigated the effects of the inhibition of GIP signaling on adiponectin levels and the stimulation of fat oxidation that averts obesity by comparing WT mice and $Gipr^{-/-}$ mice fed HFD. To clarify the early response to HFD-induced obesity, we performed the experiments on mice at 3 weeks of HFD and control-fat diet (CD).

Materials and methods

Animals. The generation of $Gipr^{-/-}$ mice (C57BL/6 background) has been described previously [11]. At 7 weeks of age, WT and $Gipr^{-/-}$ mice were fed HFD or CD for 7 weeks. HFD supplied 45% of calories as fat, 35% as carbohydrate, and 20% as protein, with energy density of 3.57 kcal/g. CD supplied 13% of calories as fat, 60% as carbohydrate, and 27% as protein, with energy density of

* Corresponding author. Address: Department of Diabetes and Clinical Nutrition, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan. Fax: +81 75 751 4244.
E-mail address: inagki@metab.kuhp.kyoto-u.ac.jp (N. Inagaki).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.08.052

3.57 kcal/g. Animal care and procedures were approved by the Animal Care Committee of Kyoto University.

Energy expenditure. Energy expenditure was evaluated by measuring respiratory quotient and oxygen consumption by indirect calorimetry every 13 min for 24 h in mice under the fed condition, as described previously [8,9,12]. Air from the room was pumped through the chamber, and expired gas was dried in a cotton thin column and subjected to gas analysis (Alco System model RL-600, Chiba, Japan). O_2 and CO_2 concentrations were measured, and oxygen consumption (VO_2), carbon dioxide exhaustion (VCO_2), respiratory quotient (RQ), and fat oxidation were calculated as described previously [12].

Computed tomography. Mice were anesthetized with pentobarbital and fixed in a chamber, and transaxially scanned using Latheta (LCT-100M) experimental animal CT system (Aloka, Tokyo, Japan). The whole body was scanned, and contiguous 1-mm slice images of the trunk were used for quantitative assessment (Latheta software, version 1.00). Weight of visceral fat mass and lean mass were quantitatively evaluated.

Measurement of plasma adiponectin levels and Western blot analysis. Blood samples were collected from the tail vein at the end of the dark phase and centrifuged (3000 rpm, 10 min, 4 °C). Levels of plasma adiponectin were measured using an adiponectin ELISA kit (Otsuka, Tokyo, Japan).

Plasma samples of HFD-fed mice were subjected to SDS–PAGE using Laemmli's method [13]. SDS–PAGE was performed under non-reducing and non-heat-denaturing conditions, as previously reported [14]. Western blot analysis was performed using anti-mouse adiponectin antibody. Densities of corresponding bands were quantified by NIH-Image.

Isolation of total RNA and quantitative RT-PCR. Total RNA was isolated from muscle and white adipose tissue (epididymal fat pad, (WAT)) using Trizol (Invitrogen, Grand Island, NY). mRNA levels were measured by real-time quantitative RT-PCR using ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). mRNA levels were corrected for GAPDH (Applied Biosystems) mRNA level. Sequences of PPARα primers were 5′-CGACCTGAAAG ATTCGGAAA-3′ and 5′-CCTCTGCCTCTTTGTCTTC-3′; sequences of ACC primers were 5′-CCTCCGAGGAACCCTCTGT-3′ and 5′-CGGCTGT CCAGTTGGTTTG-3′; sequences of adiponectin primers were 5′-G GAACTTGTGCAGGTTGGAT-3′ and 5′-GCTTCTCCAGGCTCTCCTTT-3′; and sequences of PPARγ primers were 5′-TGTCGGTTTCAGAAGT GCCTT-3′ and 5′-GCTCGCAGATCAGCAGACTCT-3′.

Statistical analysis. Data are expressed as means ± SE. Statistical analysis was performed by ANOVA and unpaired student's test. *P* values <0.05 were considered significant.

Results

Body weight and fat mass in HFD-fed Gipr$^{-/-}$ mice

WT mice exhibited significant weight gain after 3 weeks of HFD feeding (Fig. 1A). By contrast, body weight of HFD-fed Gipr$^{-/-}$ mice was not increased compared with that of CD-fed Gipr$^{-/-}$ mice after 3 weeks of study (Fig. 1B). The lesser body weight of Gipr$^{-/-}$ mice continued through 7 weeks of HFD feeding.

At the early stage of 3 weeks of HFD feeding, CT analysis was performed to estimate visceral fat mass in WT and Gipr$^{-/-}$ mice. There was no significant difference in visceral fat mass between WT and Gipr$^{-/-}$ mice under CD feeding. Visceral fat mass of HFD-fed WT and Gipr$^{-/-}$ mice were significantly increased compared with those of CD-fed WT and Gipr$^{-/-}$ mice by 100% and 52%, respectively. In HFD-fed mice, visceral fat mass of WT mice was significantly increased compared with that of Gipr$^{-/-}$ mice (Fig. 1C and D). There was no difference in lean body mass (data not shown). There also was no difference in food intake between WT and Gipr$^{-/-}$ mice (data not shown).

Fat consumption in HFD-fed Gipr$^{-/-}$ mice

To evaluate energy consumption in the early stage of HFD feeding, respiratory quotient and oxygen consumption were measured

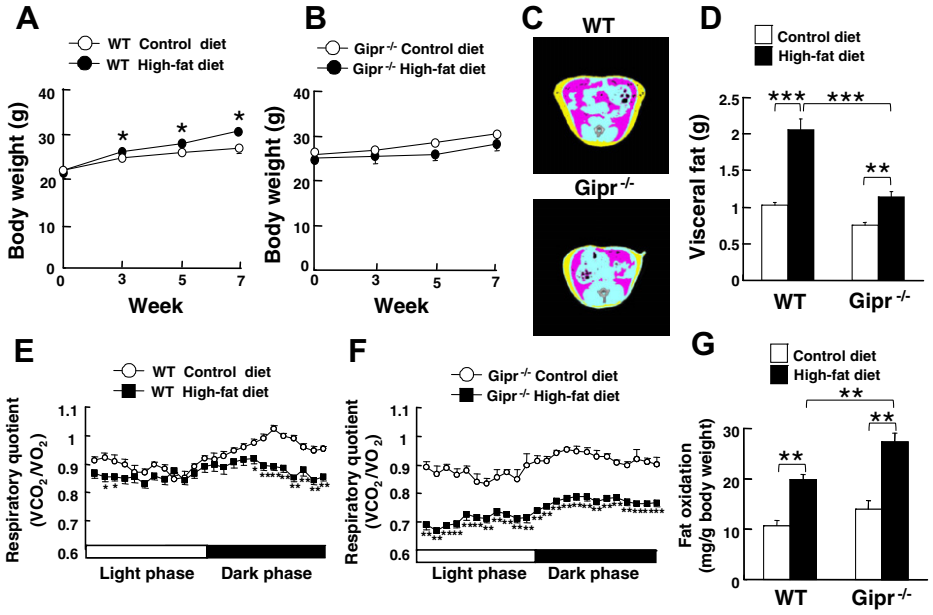

Fig. 1. Body weight in WT mice (A) and Gipr$^{-/-}$ mice (B) during 7 weeks on CD (open circles) and HFD (filled circles). (C) CT-based body composition analysis. WT mice and Gipr$^{-/-}$ mice at 3 weeks on HFD feeding. Representative CT images were taken at the same slice level. Pink, yellow, and blue areas represent visceral fat, subcutaneous fat, and lean mass. (D) Visceral fat accumulation in WT mice and Gipr$^{-/-}$ mice at 3 weeks on CD (open bars) and HFD (filled bars). Respiratory quotient in WT mice (E) and Gipr$^{-/-}$ mice (F) at 3 weeks on CD (open circles) and HFD (filled square). (G) Calculated fat oxidation in WT mice and Gipr$^{-/-}$ mice at 3 weeks on CD (open bars) and HFD (filled bars). n = 5–12. Values are means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

by indirect calorimetry. HFD-fed WT mice exhibited a significant reduction of respiratory quotient only in part of the dark phase compared with CD-fed WT mice (Fig. 1E). By contrast, HFD-fed Gipr$^{-/-}$ mice exhibited a significant reduction of respiratory quotient throughout the day compared with CD-fed Gipr$^{-/-}$ mice (Fig. 1F). At 3 weeks of HFD feeding, calculated fat oxidation was significantly increased in Gipr$^{-/-}$ mice compared with that in WT mice (Fig. 1G). These results indicate that Gipr$^{-/-}$ mice use fat as preferred energy substrate in the early stage of HFD feeding.

Adiponectin levels of Gipr$^{-/-}$ mice in the early stage of HFD feeding

We examined mRNA expression level of adiponectin in white adipose tissue (WAT) and plasma adiponectin levels in Gipr$^{-/-}$ mice. mRNA expression of adiponectin in WAT and plasma adiponectin levels were significantly increased in HFD-fed Gipr$^{-/-}$ mice compared with those in HFD-fed WT mice (Fig. 2A and B). In addition, in Gipr$^{-/-}$ mice, mRNA expression level of adiponectin in WAT and plasma adiponectin levels were significantly increased in HFD-fed mice compared with those in CD-fed mice, although HFD feeding decreased mRNA expression of adiponectin and plasma adiponectin levels in WT mice (Fig. 2A and B).

To determine qualitative differences in adiponectin, we performed Western blot analysis. Levels of middle molecular weight (MMW) and low molecular weight (LMW) multimers of adiponectin were significantly higher in HFD-fed Gipr$^{-/-}$ mice compared to those in HFD-fed WT mice (Fig. 2C and Table 1). There were no significant differences in high molecular weight multimers (HMW) of adiponectin between HFD-fed WT and Gipr$^{-/-}$ mice.

As PPARγ (peroxisome proliferator-activated receptor-γ) is known to be involved in the regulation of adiponectin, we examined mRNA expression levels of PPARγ in WAT. While HFD-fed WT mice showed a tendency to decreased mRNA expression of PPARγ, the mRNA expression level of PPARγ was significantly increased in HFD-fed Gipr$^{-/-}$ mice compared with that in HFD-fed WT mice (Fig. 2D). These results suggest that inhibition of GIP signaling modulates adiponectin levels through PPARγ.

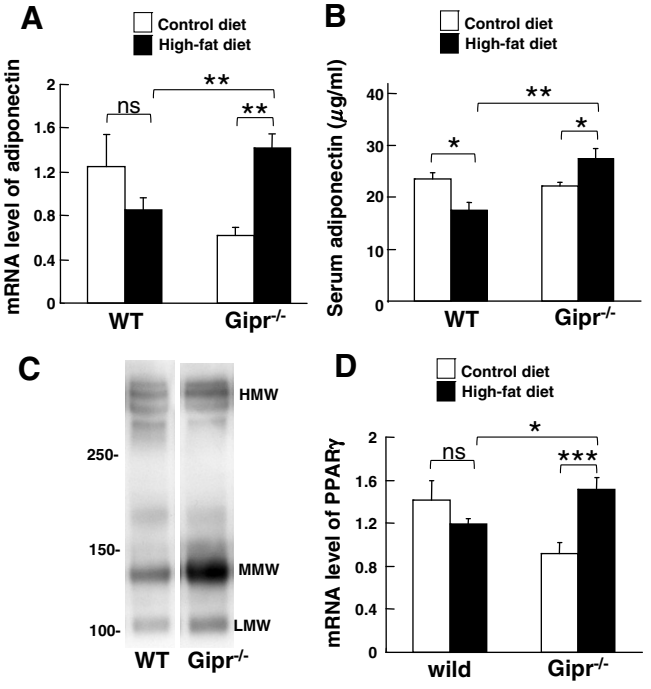

Fig. 2. mRNA expression level of adiponectin in WAT (A) and plasma adiponectin level (B) at 3 weeks on CD (open bars) and HFD (filled bars). $n = 7–8$ mice/group. Values are means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$. (C) Non-reducing and non-heat-denaturing SDS–PAGE of adiponectin in WT mice and Gipr$^{-/-}$ mice at 3 weeks on HFD. (D) mRNA expression level of PPARγ in WAT at 3 weeks on CD (open bars) and HFD (filled bars). $n = 4–7$. Values are means ± SE. $^{*}P < 0.05$, $^{***}P < 0.005$. ns, not significant.

Table 1
Difference in multimer formation of adiponectin in WT mice and Gipr$^{-/-}$ mice at 3 weeks on high-fat diet

	WT	Gipr$^{-/-}$
HMW	100 ± 22.0	131.9 ± 17.8
MMW	100 ± 9.1	154.3 ± 5.9*
LMW	100 ± 13	163.4 ± 15.3*

Values for Gipr$^{-/-}$ mice represent relative density (%) regarding average value for WT mice as 100. $n = 5$. Values are means ± SE.
* $P < 0.05$ vs. WT mice.

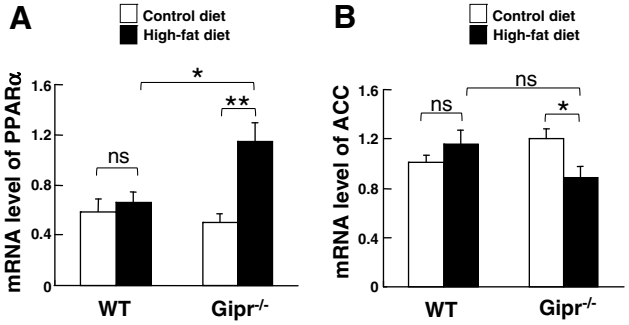

Fig. 3. mRNA expression level of PPARα (A) and ACC (B) in skeletal muscle at 3 weeks on CD (open bars) and HFD (filled bars). $n = 5–7$. Values are means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$. ns, not significant.

Expression levels of PPARα and ACC mRNAs in skeletal muscle of Gipr$^{-/-}$ mice at the early stage of HFD feeding

To determine the effects of adiponectin on peripheral tissues in the absence of GIP signaling, mRNA expression levels of PPARα (peroxisome proliferator-activated receptor-α) and ACC (acetyl-CoA carboxylase) in skeletal muscle and liver were examined. mRNA expression level of PPARα mRNA was significantly increased in HFD-fed Gipr$^{-/-}$ mice compared with that in CD-fed Gipr$^{-/-}$ mice in skeletal muscle (Fig. 3A). Although there was no significant difference in phosphorylation of AMPK (AMP-activated protein kinase) between HFD-fed WT and Gipr$^{-/-}$ mice by western blot analysis (data not shown), mRNA expression level of ACC, which is inactivated by AMPK, was significantly reduced in muscle of HFD-fed Gipr$^{-/-}$ mice compared with that in CD-fed Gipr$^{-/-}$ mice (Fig. 3B). These results indicate that fat oxidation is increased in skeletal muscle of Gipr$^{-/-}$ mice in the early stage of HFD feeding. We also examined mRNA expression levels of PPARα and ACC in liver, but no significant differences were found among the groups of mice (data not shown).

Discussion

In this study, we investigated the effects of GIP inhibition on fat oxidation in the early stage (3 weeks) of HFD feeding, and found that inhibition of GIP signaling increases the level of adiponectin, which promotes fat oxidation in peripheral tissues.

The GIP receptor is expressed in adipocytes as well as in pancreatic β-cells, and GIP signaling directly promotes energy accumulation into adipocytes. Previously, Gipr$^{-/-}$ mice were shown to exhibit resistance to high-fat-induced obesity, and to use fat as the preferred energy substrate [8]. In that study, mice were fed

HFD for a long period of from 7 to 50 weeks of age. In the present study, we investigated HFD-fed mice in the short, early period from 7 to 10 weeks of age, and found Gipr$^{-/-}$ mice to be resistant to obesity as well as to accumulation of visceral fat, and that fat oxidation was significantly increased, demonstrating that GIP plays a critical role in promoting obesity even at 3 weeks on HFD feeding.

Leptin and adiponectin are major adipokines that activate fat oxidation for body weight control. They affect AMPK activation and stimulate fat oxidation in skeletal muscle and liver [10,15]. It has been reported that inhibition of GIP signaling decreases body weight even in leptin-deficient ob/ob mice [8], indicating that factors other than leptin increase fat oxidation under conditions of inhibited GIP signaling. Leptin suppresses food intake through the central nervous system, and there were no differences in food intake between WT and Gipr$^{-/-}$ mice in CD-fed and HFD-fed mice in the present study. Thus, we focused on adiponectin, and hypothesized that inhibition of GIP signaling modulates adiponectin levels and affects on fat oxidation in peripheral tissues. The mRNA expression level of adiponectin in adipocytes and adiponectin concentrations in plasma are reduced in obese and insulin-resistant states which increases visceral fat mass [16,17]. In the present study, mRNA expression level of adiponectin in WAT and plasma adiponectin levels were found to be decreased and visceral fat mass was increased in HFD-fed WT mice compared with CD-fed WT mice. The mRNA expression and plasma levels of adiponectin were significantly increased in HFD-fed Gipr$^{-/-}$ mice compared with those in CD-fed Gipr$^{-/-}$ mice, although visceral fat mass of HFD-fed Gipr$^{-/-}$ mice was significantly increased compared with that of CD-fed Gipr$^{-/-}$ mice. These results suggest that GIP may reduce adiponectin levels in adipocytes. It has been previously reported that Gipr$^{-/-}$ mice show no difference in plasma adiponectin levels between CD-fed and HFD-fed mice after a long period of 20 weeks, when obesity is established [18]. On the other hand, we measured adiponectin levels of mice fed HFD for a short period of 3 weeks, and demonstrate that inhibition GIP signaling modulates adiponectin even in the very early stage when obesity first begins to appear in WT mice.

While skeletal muscle and liver are the major sites of body fat oxidation, the GIP receptor is not expressed in these tissues. PPARα and AMPK are the most important molecules in the control of fat oxidation in muscle and liver. Adiponectin increases expression levels of PPARα and induces phosphorylation of AMPK [16,19]. AMPK activated by adiponectin suppresses ACC activity, which catalyses the formation of malonyl-CoA and stimulates fat oxidation [20]. We found that the mRNA expression level of PPARα was significantly increased and that of ACC was significantly reduced in skeletal muscle of HFD-fed Gipr$^{-/-}$ mice compared to those in CD-fed Gipr$^{-/-}$ mice. These results indicate that fat oxidation is increased in skeletal muscle of Gipr$^{-/-}$ mice in the early stage of HFD feeding, but there was no significant difference in liver. Adiponectin has three forms: trimers (low molecular weight, LMW), hexamers (middle molecular weight, MMW), and multimers (high molecular weight, HMW), and differing tissue-specific effects of these forms on AMPK phosphorylation have been reported [21]. Only trimers activate AMPK in muscle; hexamers and the high molecular isoform do not [22]. It also has been reported that trimers are the most potent isoform in skeletal muscle [19,23]. The trimer isoform of adiponectin was found in the present study to be significantly increased in HFD-fed Gipr$^{-/-}$ mice. This finding may explain the difference of PPARα and ACC expression between skeletal muscle and liver in HFD-fed Gipr$^{-/-}$ mice.

In conclusion, we show that inhibition of GIP signaling upregulates the adiponectin level and increases fat oxidation in skeletal muscle. These findings suggest that the influence of GIP on adiposity is, at least in part, mediated by modulation of adiponectin expression in adipocytes in the early stage of HFD feeding.

Acknowledgments

We thank Drs. Takashi Kadowaki and Naoto Kubota (Department of Diabetes and Metabolic Disease, Tokyo University Graduate School of Medicine) for providing anti-mouse adiponectin antibody and for their valuable suggestion.

We also thank Drs. Katsushi Tsukiyama, Rie Watanabe, Heying Zhou, Chizumi Yamada, and Yukiko Kawasaki for their technical help and for their valuable comments.

This study was supported by Scientific Research Grants from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and from the Ministry of Health, Labor, and Welfare, Japan.

References

[1] P. Schauder, J. Brown, H. Frerichs, W. Creutzfeldt, Gastric inhibitory polypeptide: effect on glucose-induced insulin release from isolated rat pancreatic islets in vitro, Diabetologia 11 (1975) 483–484.
[2] J. Dupre, S. Ross, D. Watson, J. Brown, Stimulation of insulin secretion by gastric inhibitory polypeptide in man, J. Clin. Endocrinol. Metab. 37 (1973) 826–828.
[3] Y. Yamada, K. Miyawaki, K. Tsukiyama, N. Harada, C. Yamada, Y. Seino, Pancreatic and extrapancreatic effects of gastric inhibitory polypeptide, Diabetes 55 (Suppl. 2) (2006) S86–S91.
[4] T.B. Usdin, E. Mezey, D.C. Button, M.J. Brownstein, T.I. Bonner, Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain, Endocrinology 133 (1993) 2861–2870.
[5] R.H. Eckel, W.Y. Fujimoto, J.D. Brunzell, Gastric inhibitory polypeptide enhanced lipoprotein lipase activity in cultured preadipocytes, Diabetes 28 (1979) 1141–1142.
[6] J. Knapper, S. Puddicombe, L. Morgan, J. Fletcher, Investigations into the actions of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1(7-36)amide on lipoprotein lipase activity in explants of rat adipose tissue, J. Nutr. 125 (1995) 183–188.
[7] H. Hauner, G. Glatting, D. Kaminska, E. Pfeiffer, Effects of gastric inhibitory polypeptide on glucose and lipid metabolism of isolated rat adipocytes, Ann. Nutr. Metab. 32 (1988) 282–288.
[8] K. Miyawaki, Y. Yamada, N. Ban, Y. Ihara, K. Tsukiyama, H. Zhou, S. Fujimoto, A. Oku, K. Tsuda, S. Toyokuni, H. Hiai, W. Mizunoya, T. Fushiki, J. Holst, M. Makino, A. Tashita, Y. Kobara, Y. Tsubamoto, T. Jinnouchi, T. Jomori, Y. Seino, Inhibition of gastric inhibitory polypeptide signaling prevents obesity, Nat. Med. 8 (2002) 738–742.
[9] H. Zhou, Y. Yamada, K. Tsukiyama, K. Miyawaki, M. Hosokawa, K. Nagashima, K. Toyoda, R. Naitoh, W. Mizunoya, T. Fushiki, T. Kadowaki, Y. Seino, Gastric inhibitory polypeptide modulates adiposity and fat oxidation under diminished insulin action, Biochem. Biophys. Res. Commun. 335 (2005) 937–942.
[10] T. Kadowaki, T. Yamauchi, Adiponectin and adiponectin receptors, Endocr. Rev. 26 (2005) 439–451.
[11] K. Miyawaki, Y. Yamada, H. Yano, H. Niwa, N. Ban, Y. Ihara, A. Kubota, S. Fujimoto, M. Kajikawa, A. Kuroe, K. Tsuda, H. Hashimoto, T. Yamashita, T. Jomori, F. Tashiro, J. Miyazaki, Y. Seino, Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice, Proc. Natl. Acad. Sci. USA 96 (1999) 14843–14847.
[12] K. Ishihara, S. Oyaizu, K. Onuki, K. Lim, T. Fushiki, Chronic (−)-hydroxycitrate administration spares carbohydrate utilization and promotes lipid oxidation during exercise in mice, J. Nutr. 130 (2000) 2990–2995.
[13] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[14] H. Waki, T. Yamauchi, J. Kamon, Y. Ito, S. Uchida, S. Kita, K. Hara, Y. Hada, F. Vasseur, P. Froguel, S. Kimura, R. Nagai, T. Kadowaki, Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin, J. Biol. Chem. 278 (2003) 40352–40363.
[15] Y. Minokoshi, Y. Kim, O. Peroni, L. Fryer, C. Müller, D. Carling, B. Kahn, Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase, Nature 415 (2002) 339–343.
[16] T. Yamauchi, J. Kamon, H. Waki, Y. Imai, N. Shimozawa, K. Hioki, S. Uchida, Y. Ito, K. Takakuwa, J. Matsui, M. Takata, K. Eto, Y. Terauchi, K. Komeda, M. Tsunoda, K. Murakami, Y. Ohnishi, T. Naitoh, K. Yamamura, Y. Ueyama, P. Froguel, S. Kimura, R. Nagai, T. Kadowaki, Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis, J. Biol. Chem. 278 (2003) 2461–2468.
[17] C. Weyer, T. Funahashi, S. Tanaka, K. Hotta, Y. Matsuzawa, R. Pratley, P. Tataranni, Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia, J. Clin. Endocrinol. Metab. 86 (2001) 1930–1935.
[18] T. Hansotia, A. Maida, G. Flock, Y. Yamada, K. Tsukiyama, Y. Seino, D.J. Drucker, Extrapancreatic incretin receptors modulate glucose homeostasis, body weight, and energy expenditure, J Clin Invest 117 (2007) 143–152.
[19] T. Yamauchi, J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, Y. Mori, T. Ide, K. Murakami, N. Tsuboyama-Kasaoka, O. Ezaki, Y. Akanuma, O. Gavrilova, C.

Vinson, M. Reitman, H. Kagechika, K. Shudo, M. Yoda, Y. Nakano, K. Tobe, R. Nagai, S. Kimura, M. Tomita, P. Froguel, T. Kadowaki, The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity, Nat. Med. 7 (2001) 941–946.
[20] B. Kiens, Skeletal muscle lipid metabolism in exercise and insulin resistance, Physiol. Rev. 86 (2006) 205–243.
[21] M. Barnea, A. Shamay, A. Stark, Z. Madar, A high-fat diet has a tissue-specific effect on adiponectin and related enzyme expression, Obesity (Silver Spring) 14 (2006) 2145–2153.
[22] T. Tsao, E. Tomas, H. Murrey, C. Hug, D. Lee, N. Ruderman, J. Heuser, H. Lodish, Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity. Different oligomers activate different signal transduction pathways, J. Biol. Chem. 278 (2003) 50810–50817.
[23] J. Fruebis, T. Tsao, S. Javorschi, D. Ebbets-Reed, M. Erickson, F. Yen, B. Bihain, H. Lodish, Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice, Proc. Natl. Acad. Sci. USA 98 (2001) 2005–2010.