Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 341 (2006) 209–217
BBRC
www.elsevier.com/locate/ybbrc

Changes of skeletal muscle adiponectin content in diet-induced insulin resistant rats

Baichun Yang *, Lihong Chen, Ying Qian, James A. Triantafillou, Judi A. McNulty, Kevin Carrick, Lisa G. Clifton, Bajin Han, Robert Geske, Jay Strum, Kathleen K. Brown, Stephen A. Stimpson, Greg Pahel

GlaxoSmithKline, Research Triangle Park, NC 27709, USA

Received 23 December 2005
Available online 9 January 2006

Abstract

The current study examined the relationship between skeletal muscle levels of adiponectin and parameters of insulin sensitivity. A high fat/sucrose diet (HFD) for 20 weeks resulted in significant increases in body weight, serum insulin, triglycerides (TG), and free fatty acids (FFA) (all $p < 0.01$). Interestingly, this diet leads to a slight increase in serum adiponectin, but significant decreases in gastrocnemius muscle and white adipose adiponectin (all $p < 0.05$). HFD for 4 weeks also resulted in a significant decrease in muscle adiponectin, which correlated with serum insulin, TG, and FFA (all $p < 0.05$). Treatment of the 4-week HFD rats with a PPARγ agonist GI262570 ameliorated the diet-induced hyperinsulinemia and dyslipidemia, and effectively restored muscle adiponectin (all $p < 0.05$). This study demonstrated that HFD-induced hyperinsulinemia and dyslipidemia appeared without changes in serum adiponectin, but were associated with decreased tissue adiponectin. This provides the first evidence for a connection between tissue adiponectin and diet-induced hyperinsulinemia and dyslipidemia.

Keywords: Adiponectin; Insulin; Muscle; Liver; White adipose tissue PPARγ; Lipid; Rat

The adipokine, adiponectin, has been shown to have insulin-sensitizing, anti-atherogenic, and anti-inflammatory properties [1]. Studies by Maeda et al. [2] showed that adiponectin knockout mice developed hyperglycemia and hyperinsulinemia while on high fat diet, which was reversed by adenoviral-adiponectin expression. Administration of adiponectin caused glucose-lowering effects and ameliorated insulin resistance in mice [3,4]. The insulin-sensitizing effect of adiponectin was reported to be mediated by an increase in fatty acid oxidation through activation of AMP-activated protein kinase (AMPK) [5,6], a decreased hepatic glucose production during a pancreatic euglycemic clamp [7], and an increased post-absorptive insulin-mediated suppression of hepatic glucose output [1].

It has been well documented that plasma adiponectin is lower in obese subjects than in lean human subjects, lower in diabetic patients than in non-diabetic patients [1,8–10], and is negatively correlated with body weight, visceral fat mass, and insulin level [8,9]. A study by Tschritter et al. [11] in non-diabetic individuals analyzed the associations between plasma adiponectin and insulin sensitivity and serum lipid parameters, and concluded that plasma adiponectin predicts insulin sensitivity of both glucose and lipid metabolism. Several studies have shown that in humans low adiponectin levels predict future insulin resistance [12], hyperglycemia [13], and diabetes [14–16].

Although adiponectin levels clearly correlate with body mass index (BMI) in every study, the predictive value of adiponectin with regard to future obesity [17] and the relationship of adiponectin with changes in body weight are not so clear. Weight loss due to bariatric surgery [18],

* Corresponding author. Fax: +1 919 483 3731.
E-mail address: baichun.w.yang@gsk.com (B. Yang).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.12.172

caloric restriction [19], or a long-term weight management program in women [20] does result in increased circulating levels of adiponectin. The situation with regard to weight gain is less clear. A study in mice on a high fat (36% lard) diet found no change in serum adiponectin levels [21]. In several diet-induced obesity studies in mice, adiponectin mRNA expression in WAT was unchanged in one [22], while in three others it was reduced [23–25].

Our own recently reported studies [26] in male SD rats showed that high fat/sucrose diet for 2–4 weeks led to hyperinsulinemia and dyslipidemia, but a slightly higher level of serum adiponectin. Hotta et al. [27] have reported that adiponectin decreased in parallel with the progression of type 2 diabetes (T2D) in rhesus monkeys with a strong correlation between plasma adiponectin and systemic insulin sensitivity. Insulin-sensitizing PPARγ agonists have a marked effect of up-regulating serum adiponectin. Combs et al. [28] reported that PPARγ agonist rosiglitazone increased plasma adiponectin in *db*/*db* mice. Yang et al. [29] reported rosiglitazone-increased plasma levels of adiponectin in T2D patients.

Skeletal muscle is the most important organ for energy expenditure and is one of the major sites for insulin action [30]. T2D is characterized by the resistance of peripheral tissues, including skeletal muscle, liver, and adipose, to the action of insulin [31]. AMPK is an enzyme that has been shown to increase muscle fatty acid oxidation and insulin sensitivity [32]. Tomas et al. [6] reported that, in both in vivo and in vitro models, globular adiponectin led to increases in AMPK activity and phosphorylation of both AMPK and acetyl CoA carboxylase (ACC), and in 2-deoxyglucose uptake in skeletal muscles. Yamauchi et al. [5] showed that globular and full-length adiponectin stimulated phosphorylation and activation of AMPK in skeletal muscle and stimulated phosphorylation of ACC, fatty acid oxidation, glucose uptake, and lactate production in myocytes. In cultured myocytes, TNF-α decreased fatty acid transport protein 1 mRNA expression, insulin-receptor substrate 1 (IRS-1)-associated phosphatidylinositol 3 kinase (PI3) activity, and glucose uptake, whereas adiponectin increased these parameters [2]. For these reasons, skeletal muscle is one of the most important sites of action for adiponectin as well as insulin, and alterations of tissue adiponectin level in skeletal muscle could be important to the early stage of insulin resistance.

The present studies were designed to examine the effect of longer-term high fat diet on adiponectin levels in rats and to explore the involvement of skeletal muscle tissue adiponectin level in the sub-acute high fat/sucrose diet-induced hyperinsulinemia and dyslipidemia in male SD rats, and the effect of PPARγ agonists.

Methods

Experimental animal and protocols. All procedures performed were in compliance with the Animal Welfare Act and U.S. Department of Agriculture regulations, and were approved by the GlaxoSmithKline Animal Care and Use Committee. Male cesarian-derived Sprague–Dawley rats (SD, 225–250 g) (Charles River, Indianapolis, IN) were fed a rodent regular chow Purina 5001 (Harlan Teklad, Indianapolis, IN). All studies were conducted after 1-week adaptation period. For the first set long-term diet study, SD rats were fed a high fat/sucrose diet (TD88137, Harlan Teklad, Indianapolis, IN, containing 34.146% sucrose, 42% of calories from fat) or chow Purina 5001 (served as normal diet control) for 20 weeks ($n = 9$/group). For the second set short-term diet study, SD rats were fed the high fat/sucrose or a regular chow diet for 4 weeks. SD rats on the 4-week high fat/sucrose diet were treated with vehicle (0.5% hydroxypropyl methylcellulose and 0.1% Tween 80), PPARγ agonist GI262570 [33,34] (0.2, 2, 20, or 100 mg/kg, QD), for the last 2 weeks. Tail vein bleeding was performed in all rats between 9 and 11 am at day 0, day 2, week 2, 4, 8, 14, and 20, under isoflurane (Abbott Laboratories, IL). Body weight was also recorded. Serum was obtained for determining glucose, insulin, triglycerides (TG), free fatty acids (FFA), leptin, and adiponectin. White adipose tissue (WAT, epididymal fat pad), liver, and gastrocnemius muscle were collected from rats (under isoflurane) in the first set 20-week diet study and stored in −80 °C for determining adiponectin content. In the second set 4-week diet study, whole body fat mass was determined at day 0 and week 4 by DexScan; hind limb muscle was saved for determining adiponectin level and location; and WAT was saved for determining mRNA levels of PPARγ response gene FABP3. At the end of the study, all rats were euthanized with CO_2.

Determination of body fat mass by DexScan. Whole body fat mass of rats in normal diet controls and high fat/sucrose diet alone group was determined by using Hologic QDR-4500A (Hologic, MA). Briefly, Hologic QDR-4500A was calibrated with Small Animal Step Phantomn prior to body composition measurement. Rats were anesthetized with isoflurane, and placed on the densitometer table in the prone position with their spines parallel to the long axis of the table, and tails bent around their bodies. The laser beam scan was positioned approximately 1 inch posterior to tail insertion. Rats were scanned with Small Animal Rat Whole Body software (Hologic, MA). Visible analysis was then done with the global region of interest including the entire animal.

Determination of postprandial serum chemicals and leptin. Serum glucose, TG, and FFA were measured using Ilab600 Clinical Chemistry System (Instrumentation Laboratory). Leptin was determined by using TiterZyme EIA for rat leptin (Assay Designs, Ann Arbor, MI), according to the manufacturer's instruction.

Determination of serum and tissue adiponectin. Serum adiponectin of rats in short-term set was determined by using adiponectin RIA kit (Linco Research, MO), according to the manufacturer's instruction. Adiponectin in other serum and tissue samples was determined by using adiponectin ELISA kit (B-Bridge International, CA), according to the manufacturer's instruction. Hind limb muscle, liver, and WAT samples were homogenized in sample diluent of the ELISA kit (added with 5 μM leupeptin, 1 mM PMSF, and 0.05% protease inhibitor cocktail from Sigma) at 100 mg/ml. The homogenates were centrifuged at 9000 rpm, for 10 min at 4 °C. Supernatants were collected and analyzed for adiponectin.

Determination of insulin level. Serum insulin level was determined using Rat Insulin ELISA kit (Crystal Chem, IL), according to the manufacturer's instruction.

Localization of adiponectin in hind limb muscle tissues by immunohistochemical (IHC) stain. The muscle (gastrocnemius) samples were fixed in 10% buffered formalin for 24 h, transferred into 70% alcohol for several days, and then embedded in paraffin. Four-micron sections were prepared. All IHC assays were run on a Ventana Discovery System (Ventana Medical Systems) until counterstaining. Normal goat serum was used as the background blocker. Primary antibody rabbit anti-mouse adiponectin was applied onto the sections for 6 h. Pre-immune serum was used at the same concentration as primary antibodies for negative controls. Biotinylated goat anti-rabbit IgG was used as the second antibody. The sections were then visualized by DAB staining (DAB Map Kit from Ventana) and then counterstained with hematoxylin/bluing. Pre-incubation of the primary antibody with adiponectin-relevant peptide at 1:1 for 2 h completely blocked the IHC signal, indicating the specific reactivity with the primary antibody.

Determination of FABP3 mRNA level in white adipose tissue by real-time PCR. Total RNA in epididymal fat pad was isolated by the TRIZOL method [35]. All RNA samples were DNased using the DNA-*free* kit (Ambion—according to protocol). The samples were then converted to cDNA using the High Capacity cDNA Archive Kit (Applied Biosystems—according to protocol). Samples were diluted to a final concentration of 5 ng/μl cDNA. PCR results were generated using the 5′ nuclease assay (TaqMan) [36] and the ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). Primers and probe for FABP3 are: forward-GTCGTGACACTGGACGGAGG; reverse-TTCCCATCACTTAGTTCCCGTG; probe-CAGAAGTGGGACGGGCAGGAGACTACG. The primers and probe for internal control gene cyclophilin are: forward-TATCTGCACTGCCAAGACTGA; reverse-CCACAATGCTCATGCCTTCTTTCA; probe-CCAAAGACCACATGCTTGCCATCCA.

Statistical analysis. There were 7–9 rats for each data point. Data are presented as means ± SEM. Correlation between two parameters and the significant level of correlation were analyzed by Pearson correlation analysis. Differences between vehicle and treated groups were analyzed by two-way ANOVA. *P* value less than 0.05 was taken to be significant.

Results

Long-term diet study

Rats on the high fat/sucrose diet had significantly higher body weight, serum postprandial insulin, TG, FFA, and leptin than those on normal diet (Table 1 and Fig. 1). Changes in triglycerides, free fatty acids appeared after 2 days of diet. The other differences appeared 2–4 weeks after starting the diet. All these differences remained for the whole diet period. There was not any difference in serum glucose between rats on normal diet and high fat/sucrose diet (Table 1). During first 2 months, serum adiponectin was similar in both normal diet group and high fat/sucrose group. Interestingly, serum adiponectin seemed to be slightly higher in the high fat/sucrose group than in the normal diet group, the difference reaching statistical significance at week 14 (Table 1). However, at the end of 20-week diet period, tissue adiponectin content in WAT and gastrocnemius was significantly lower in rats on the high fat/sucrose diet than rats on the normal diet; tissue adiponectin content in liver was similar in both groups of rats (Fig. 2).

Short-term diet study

Similar to the long-term diet study, rats fed the high fat/sucrose diet for 4 weeks also showed significantly higher postprandial serum levels of insulin, TG, and FFA, without changes in postprandial serum levels of glucose and adiponectin (Table 2). Rats on normal diet for 4 weeks showed only a slight increase in whole body fat mass (6.67 ± 0.37% vs. 8.00 ± 0.59% of body weight, p = NS). Rats on the 4-week high fat/sucrose diet had a marked

Table 1
High fat/sucrose diet in rats for 20 weeks resulted in higher level of postprandial serum insulin, TG, FFA without change in glucose, and slightly higher level of adiponectin (n = 8–10/data point, except n = 3 at day 2)

Time	Insulin (ng/ml)		TG (mg/dL)		FFA (mEq/L)		Glucose (mg/dL)		Adiponectin (μg/ml)	
	Control	Diet	Control	Diet	Control	Diet	Control	Diet	Control	Diet
Day 0	1.1 ± 0.2	1.1 ± 0.2	70 ± 10	82 ± 12	0.17 ± 0.02	0.22 ± 0.04	178 ± 4	173 ± 3	5.0 ± 0.6	4.4 ± 0.5
Day 2	0.9 ± 0.1	1.1 ± 0.2	108 ± 26	338 ± 62*	0.19 ± 0.03	0.35 ± 0.01*	161 ± 1	164 ± 4	3.7 ± 0.6	4.4 ± 0.7
Week 2	0.9 ± 0.1	1.8 ± 0.2**	105 ± 10	569 ± 66**	0.29 ± 0.03	0.50 ± 0.03**	167 ± 4	163 ± 3	5.2 ± 0.4	5.6 ± 0.6
Week 4	1.1 ± 0.1	2.5 ± 0.3**	107 ± 12	399 ± 59**	0.34 ± 0.04	0.46 ± 0.04**	171 ± 4	173 ± 3	5.8 ± 0.4	6.6 ± 0.9
Week 8	1.6 ± 0.1	3.1 ± 0.2**	121 ± 13	428 ± 52**	0.34 ± 0.02	0.46 ± 0.02**	170 ± 4	172 ± 4	5.6 ± 0.7	7.5 ± 0.6
Week 14	1.7 ± 0.3	4.2 ± 0.6**	123 ± 12	260 ± 36**	0.29 ± 0.03	0.45 ± 0.04**	167 ± 5	179 ± 6	3.7 ± 0.3	4.9 ± 0.4*
Week 20	2.6 ± 0.3	3.5 ± 0.4**	120 ± 15	254 ± 19**	0.29 ± 0.03	0.65 ± 0.02**	188 ± 8	182 ± 6	4.8 ± 0.4	5.4 ± 0.3*

* $p < 0.5$ vs control.
** $p < 0.01$ vs control.

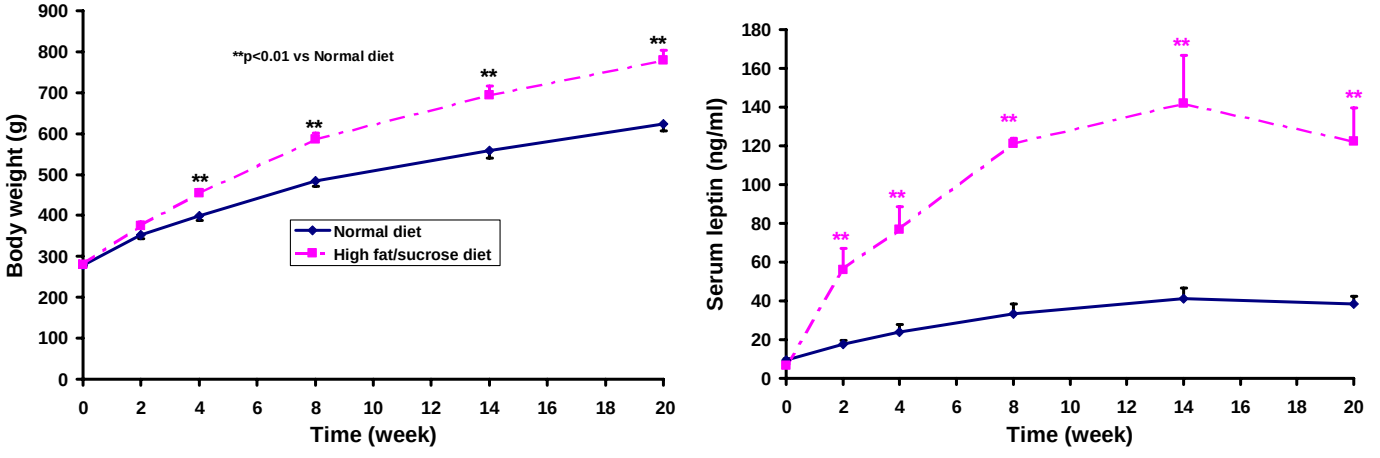

Fig. 1. Compared with rats in normal diet, high fat/sucrose diet in SD rats led to significantly higher body weight and serum leptin. The changes reached statistical significance after 2–4 weeks of diet and were sustained during the diet period (n = 9).

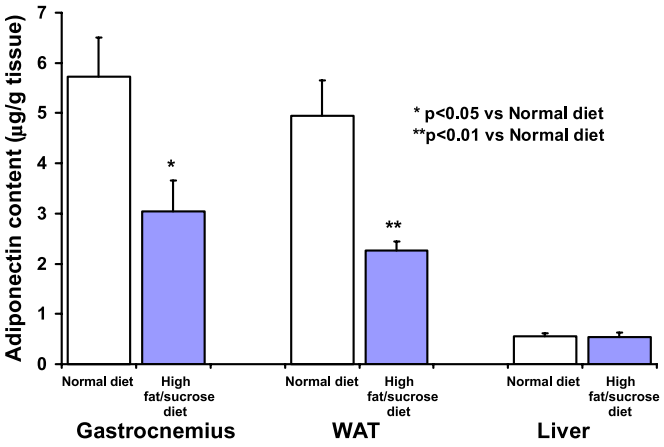

Fig. 2. Adiponectin content in skeletal muscle (gastrocnemius), WAT, and liver after 20 weeks of high fat/sucrose diet. Compared with rats on normal control diet, rats on high fat/sucrose diet had markedly lower adiponectin content in gastrocnemius and WAT. Liver adiponectin content of rats on normal diet or high fat/sucrose diet was similar ($n = 9$).

increase in whole body fat mass ($5.84 \pm 0.47\%$ vs. $17.30 \pm 1.30\%$ of body weight, $p < 0.01$). Thus, the whole body fat mass in the high fat/sucrose diet group, after 4 weeks of diet, is ~2.2 times of that in normal diet group ($p < 0.01$). Interestingly, hind limb muscle tissue adiponectin content was significantly lower in rats on the high fat/sucrose diet than rats on the normal diet (Fig. 3). The hind limb muscle adiponectin was negatively correlated with postprandial serum insulin, TG, and FFA (r: −0.4273, −0.5673, and −0.6434, respectively; all $p < 0.05$, Fig. 4). Treatment of rats on HFD diet with GI262570 (0.2, 2, 20, or 100 mg/kg/day) for the later 2 weeks effectively restored hind limb adiponectin with maximal effect at 20 mg/kg/day (Fig. 3). GI262570 treatment also dose-dependently increased serum adiponectin and FABP3 mRNA levels in WAT, which confirmed in vivo PPARγ activation and did not reach maximal effect even at 100 mg/kg/day (Fig. 3). GI262570 also diminished the high fat/sucrose diet-elevated postprandial serum insulin, TG, and FFA (Table 2). Up to the maximal efficacious dose on hind limb muscle adiponectin (20 mg/kg/day), muscle tissue adiponectin in the GI262570-treated rats was also negatively correlated with insulin, TG, and FFA (r: −0.5738, −0.4891, and −0.3453, respectively; all $p < 0.05$, Fig. 5).

Immunohistochemical staining of gastrocnemius with anti-adiponectin antibody revealed that adiponectin was localized in vascular endothelium and white adipocytes between the epimysiums of the skeletal muscle, and on the perimysium and epimysium of the muscle. The muscle cells showed no stain. There was no staining difference for adiponectin among normal diet, high fat/sucrose diet, and diet plus GI262570 groups. There was an increase in adipocyte content of muscle in rats on high fat/sucrose diet (Fig. 6).

Discussion

The adipocyte-secreted plasma protein adiponectin has gathered broad interest and attention in the area of obesity and diabetes in recent years. Experimental studies indicated its potential insulin-sensitizing and anti-atherogenic properties [1]. It has been well documented that obese and T2D patients have lower levels of plasma adiponectin [1,8–10]. Hypoadiponectinemia has been suggested to be involved in the development of insulin resistance and hyperinsulinemia [10,27,37,38]. However, all these data are from humans, long-term chronic animal studies, and genetically obese rodents, representing cumulative changes over long time periods. There is evidence that weight loss can lead to increases in circulating adiponectin [18–20], but the inverse study of weight gain in humans would be ethically challenging. Such studies in rodents have shown that long-term weight gain does not lead to decreased serum adiponectin levels. A study by Naderali et al. [39] showed that 2 days feeding on a fat/glucose-enriched diet resulted in higher serum lipid and lower plasma adiponectin, and that 16 weeks on the diet increased body weight and fat pad mass, but did not decrease serum adiponectin. This is consistent with other studies in rodents, including our own earlier report [21,26]. There is one study in rhesus monkeys reporting that adiponectin levels did decrease during weight gain-induced progression to type 2 diabetes with a strong correlation between adiponectin levels and insulin sensitivity [27]. The present studies observed the

Table 2
Effect of 4-week high fat/sucrose diet and GI262570 on postprandial serum insulin, TG, FFA, and glucose ($n = 8$/group)

Group	Insulin (ng/ml)	TG (mg/dL)	FFA (mEq/L)	Glucose (mg/dL)
Normal diet	1.23 ± 0.22	122 ± 9	0.24 ± 0.02	177 ± 3
High fat/sucrose diet				
Vehicle	$2.39 \pm 0.35^{**}$	$388 \pm 44^{**}$	$0.67 \pm 0.06^{**}$	167 ± 4
GI262570 (mg/kg/day)				
0.2	1.82 ± 0.17	418 ± 47	0.53 ± 0.05	195 ± 8
2.0	$1.48 \pm 0.07^{+}$	$257 \pm 26^{+}$	$0.31 \pm 0.03^{++}$	162 ± 6
20	$1.41 \pm 0.19^{+}$	$124 \pm 17^{++}$	$0.37 \pm 0.04^{++}$	160 ± 6
100	$1.41 \pm 0.19^{++}$	$101 \pm 15^{++}$	$0.37 \pm 0.04^{++}$	152 ± 3

** $p < 0.01$ vs control.
+ $p < 0.05$ vs vehicle.
++ $p < 0.01$ vs vehicle.

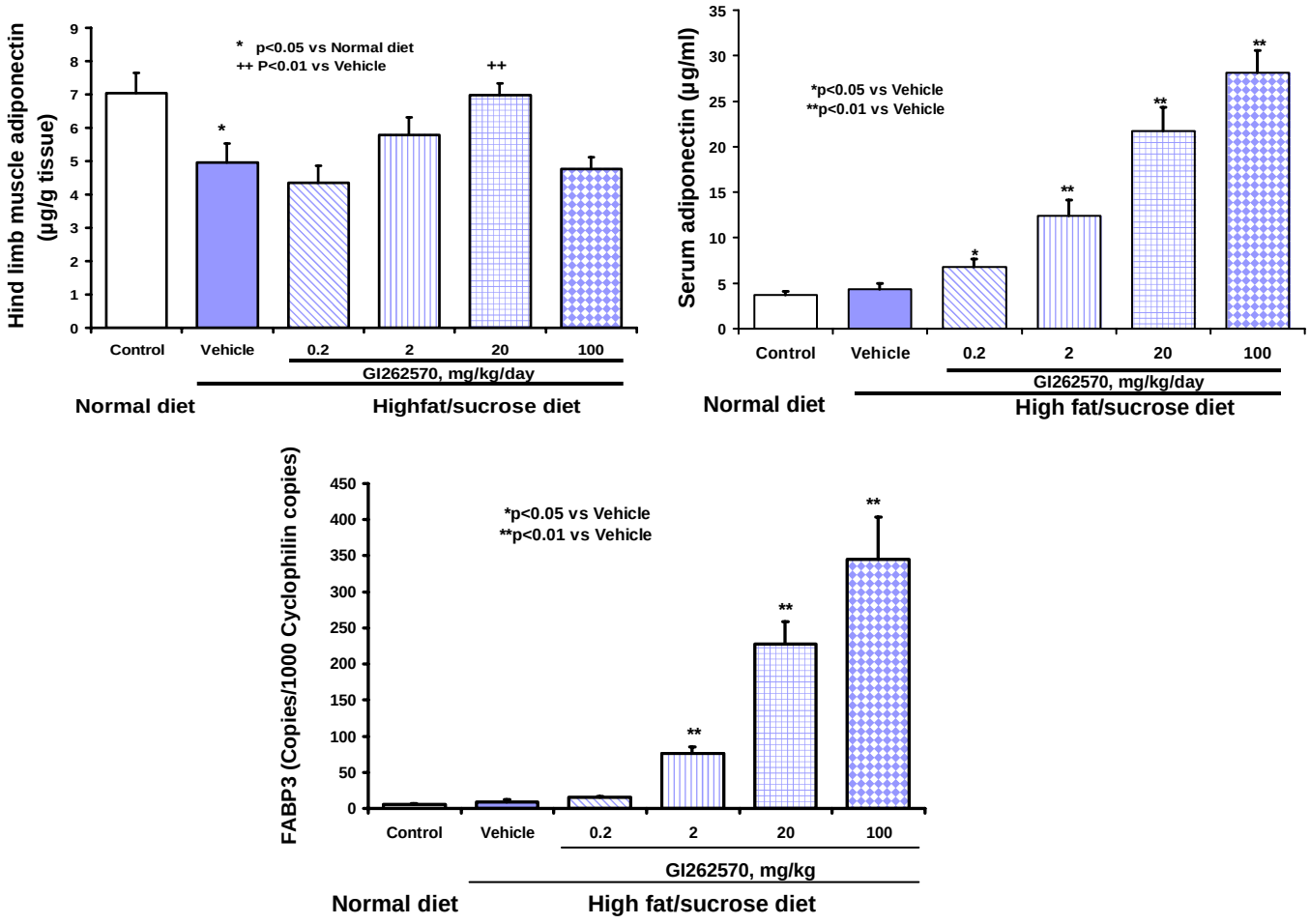

Fig. 3. Effects of high fat/sucrose diet and PPARγ agonist GI262570 on adiponectin levels of serum (up-right) and hind limb muscle (up-left), and FABP3 mRNA level in white adipose tissue ($n = 5$–8). High fat/sucrose diet for 4 weeks had no effect on serum adiponectin level, but significantly decreased hind limb muscle tissue adiponectin content. Treatment with GI262570 for the later 2 weeks elevated serum adiponectin in a dose-dependent manner and restored hind limb muscle tissue adiponectin content. The different dose–response curves for serum adiponectin and muscle adiponectin indicate that the muscle adiponectin is not from the contamination of serum adiponectin. GI262570 up-regulated FABP3 mRNA in WAT, indicating in vivo PPARγ activation.

changes during 20 weeks of high fat/sucrose diet, and found that serum adiponectin was slightly higher (instead of lower) in rats on diet, and that hyperinsulinemia and insulin resistance appeared before any significant changes in serum adiponectin, consistent with the previous reports in rodents. These findings indicate that change in circulating adiponectin is not involved in the development of insulin resistance and hyperinsulinemia in rodents, calling into question the direct involvement of adiponectin in insulin sensitization. One way to restore this direct connection between adiponectin and insulin sensitivity and to reconcile the rodent and monkey results would be if the tissue levels of adiponectin were not always directly related to the circulating levels.

Adiponectin is specifically secreted from adipocytes. Adipose mass would affect adiponectin circulating level. The present studies showed that WAT adiponectin content in long-term high fat/sucrose diet group was only ~46% of that in control diet group, while our short-term diet study also showed that fat mass in the high fat/sucrose diet group was ~2.2 times of that in control diet group [26]. It is possible that the diet-increased fat mass balances the reduced adiponectin secretion from adipocytes, with the result that the circulating level of adiponectin in the present study did not go down after 20 weeks on a high fat/sucrose diet.

Yamauchi et al. [40] cloned adiponectin receptors 1 and 2 (AdipoR1 and AdipoR2). These authors reported that AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in liver. This study concluded that AdipoR1/R2 served as receptors for globular and full-length adiponectin, and that the receptors mediated the increased AMP kinase and PPARα ligand activities, as well as fatty acid oxidation and glucose uptake by adiponectin [42]. Skeletal muscle is an important site for insulin action [30], including fatty acid oxidation and glucose uptake [32]. High fat diet and/or obesity result in decreased insulin sensitivity and lead to insulin resistance-hyperinsulimemia-T2D. Adiponectin increases insulin sensitivity and ameliorates insulin resistance via activation of AdipoR1 in skeletal muscle [40]. Therefore, alterations of

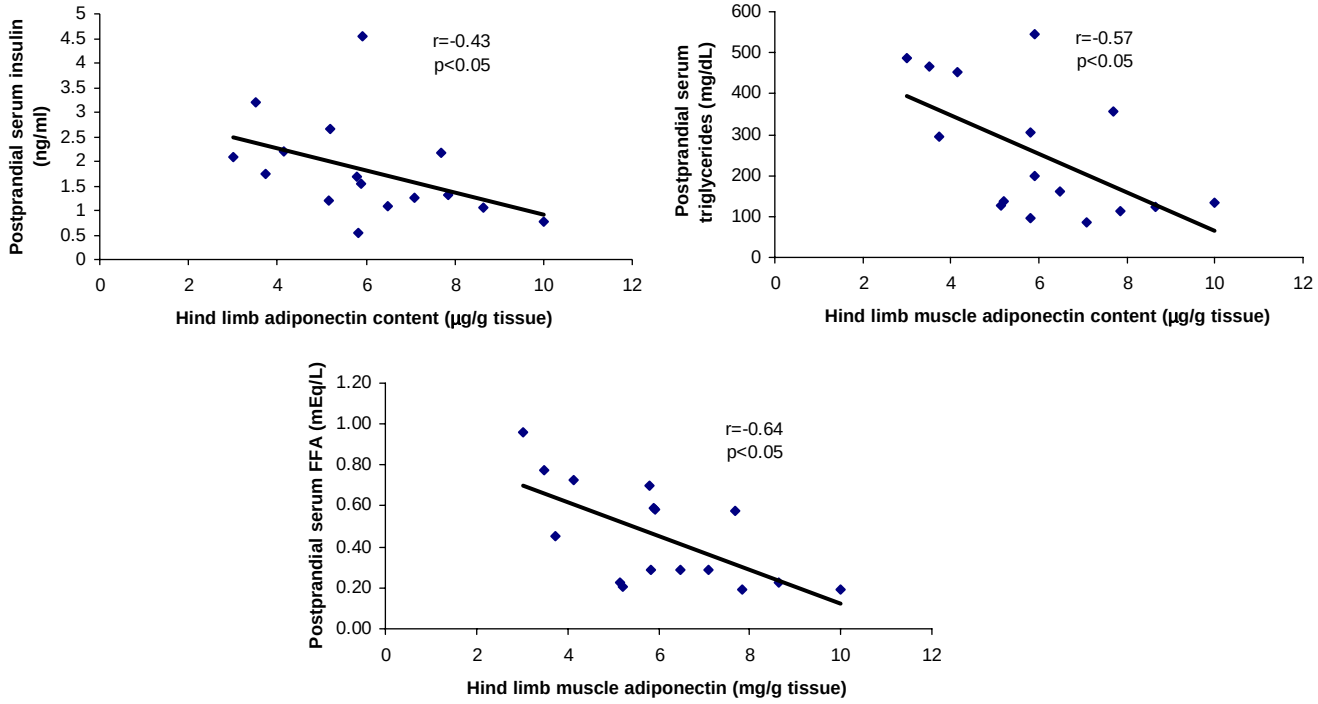

Fig. 4. Correlation for hind limb muscle adiponectin vs postprandial serum insulin, triglycerides, and free fatty acids in rats on both normal diet and high fat/sucrose diet. Hind limb muscle adiponectin content is negatively correlated with all postprandial serum insulin, triglycerides, and free fatty acids.

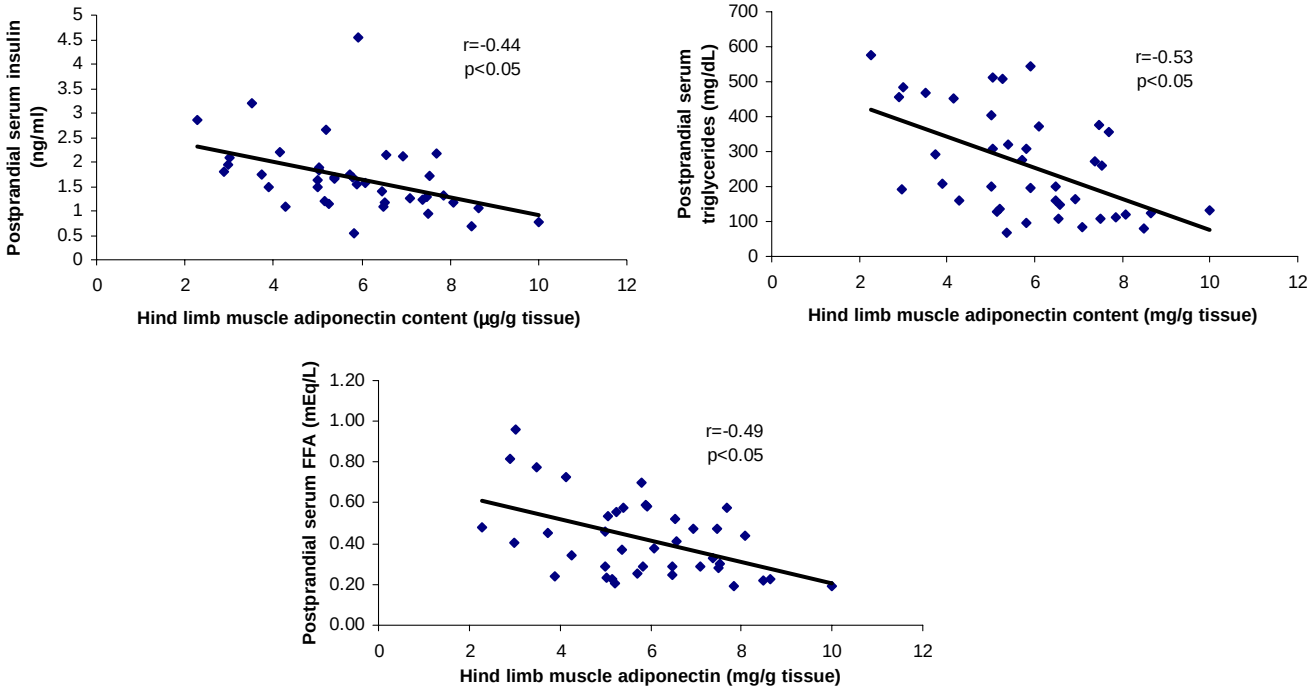

Fig. 5. Correlation for hind limb muscle adiponectin vs postprandial serum insulin, triglycerides, and free fatty acids in rats on high fat/sucrose diet and treated with GI262570 0–20 mg/kg/day. Hind limb muscle adiponectin content is negatively correlated with all postprandial serum insulin, triglycerides, and free fatty acids.

adiponectin level in skeletal muscle would be more critical in development of insulin resistance than that of serum level of adiponectin. The present study showed that rat serum adiponectin was first unchanged, then slightly increased over 20 weeks of high fat/sucrose diet, but adiponectin level in skeletal muscle tissues was markedly decreased after 4 weeks of high fat/sucrose diet. Interestingly, the present study also discovered that PPARγ agonist GI262570 resulted in increases in serum adiponectin and skeletal muscle tissue adiponectin in different dose–response styles. The dose-dependent increase in serum adiponectin did not reach a maximum at a dose of 100 mg/kg/

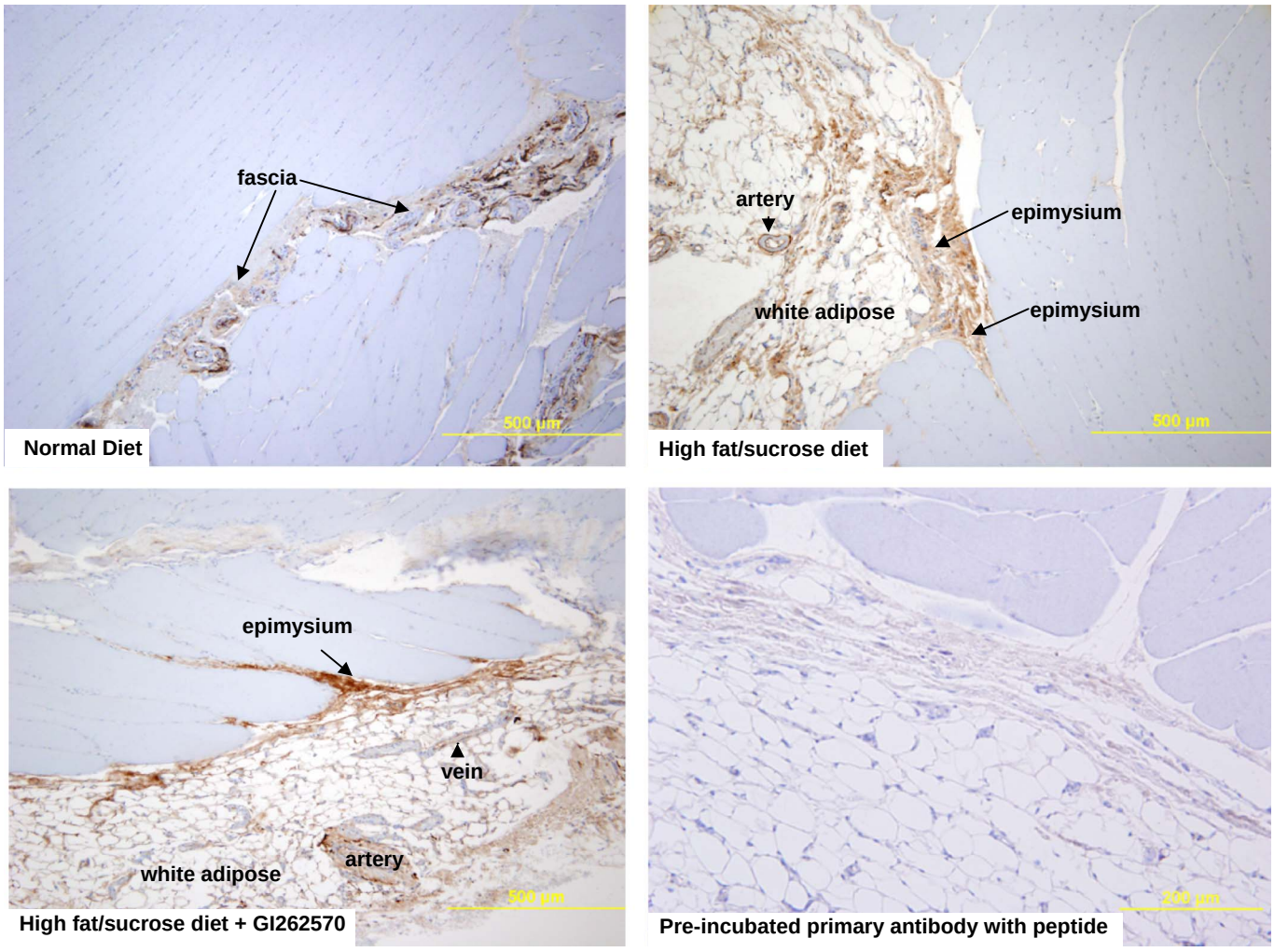

Fig. 6. Immunohistochemical staining of gastrocnemius for adiponectin and adipocytes. Adiponectin was localized in vascular endothelium and adipocytes between epimysiums, on epimysium and perimysium of the muscle. There was an increase in adipocyte content of muscle tissue in the high fat/sucrose diet groups.

day, whereas the dose-dependent preservation in skeletal muscle tissue adiponectin reached maximal effect at 20 mg/kg/day (~10 times of clinical efficacious dose) [33]. These findings indicate that the level of adiponectin measured in the muscle is simply not due to contaminating blood and that the tissue levels of adiponectin are relevant to the development of insulin resistance.

Adiponectin circulates as a homotrimer, hexamer, and even larger multimers, known as the high molecular weight (HMW) form. Various studies have suggested that the different forms of adiponectin have different activities [10,41] and even that the HMW form may be most relevant to its biological activities [42–44]. In the present study, within the limits of non-denaturing gel electrophoresis, the high fat/sucrose diet did not change the distribution of adiponectin multimers in either serum or tissue samples (data not shown).

In summary, the present study demonstrated that the decrease in skeletal muscle tissue adiponectin content and increases in serum insulin and lipids happened without any significant change in serum adiponectin; GI262570 preserved skeletal muscle adiponectin, increased serum adiponectin, and normalized serum insulin and lipids. The present study provides the first evidence for a connection between skeletal muscle tissue adiponectin content and diet-induced insulin resistance and dyslipidemia.

References

[1] A.H. Berg, T.P. Combs, P.E. Scherer, ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism, Trends Endocrinol. Metab. 13 (2002) 84–89.

[2] N. Maeda, I. Shimomura, K. Kishida, H. Nishizawa, M. Matsuda, H. Nagaretani, N. Furuyama, H. Kondo, M. Takahashi, Y. Arita, R. Komuro, N. Ouchi, S. Kihara, Y. Tochino, K. Okutomi, M. Horie, S. Takeda, T. Aoyama, T. Funahashi, Y. Matsuzawa, Diet-induced insulin resistance in mice lacking adiponectin/ACRP30, Nat. Med. 8 (2002) 731–737.

[3] J. Fruebis, T.S. Tsao, S. Javorschi, D. Ebbets-Reed, M.R. Erickson, F.T. Yen, B.E. Bihain, H.F. 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.
[4] 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.L. 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.
[5] T. Yamauchi, J. Kamon, Y. Minokoshi, Y. Ito, H. Waki, S. Uchida, S. Yamashita, M. Noda, S. Kita, K. Ueki, K. Eto, Y. Akanuma, P. Froguel, F. Foufelle, P. Ferre, D. Carling, S. Kimura, R. Nagai, B.B. Kahn, T. Kadowaki, Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase, Nat. Med. 8 (2002) 1288–1295.
[6] E. Tomas, T.S. Tsao, A.K. Saha, H.E. Murrey, C.C. Zhang, S.I. Itani, H.F. Lodish, N.B. Ruderman, Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation, Proc. Natl. Acad. Sci. USA 99 (2002) 16309–16313.
[7] T.P. Combs, A.H. Berg, S. Obici, P.E. Scherer, L. Rossetti, Endogenous glucose production is inhibited by the adipose-derived protein Acrp30, J. Clin. Invest. 108 (2001) 1875–1881.
[8] K. Hotta, T. Funahashi, Y. Arita, M. Takahashi, M. Matsuda, Y. Okamoto, H. Iwahashi, H. Kuriyama, N. Ouchi, K. Maeda, M. Nishida, S. Kihara, N. Sakai, T. Nakajima, K. Hasegawa, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Hanafusa, Y. Matsuzawa, Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 1595–1599.
[9] K. Hotta, Y. Matsuzawa, Molecular mechanism in the development of the complications associated with obesity–the physiological and pathological role of adipocytokines, Jpn. J. Clin. Med. 59 (2001) 481–486.
[10] Y. Arita, S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi, Y. Matsuzawa, Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity, Biochem. Biophys. Res. Commun. 257 (1999) 79–83.
[11] O. Tschritter, A. Fritsche, C. Thamer, M. Haap, F. Shirkavand, S. Rahe, H. Staiger, E. Maerker, H. Haring, M. Stumvoll, Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism, Diabetes 52 (2003) 239–243.
[12] Y. Yamamoto, H. Hirose, I. Saito, K. Nishikai, T. Saruta, Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: two-year follow-up study in Japanese population, J. Clin. Endocrinol. Metab. 89 (2004) 87–90.
[13] F. Fumeron, R. Aubert, A. Siddiq, D. Betoulle, F. Péan, S. Hadjadj, J. Tichet, E. Wilpart, M. Chesnier, B. Balkau, P. Froguel, M. Marre, For the epidemiologic data on the insulin resistance syndrome (DESIR) study group: adiponectin gene polymorphisms and adiponectin levels are independently associated with the development of hyperglycemia during a 3-year period. The epidemiologic data on the insulin resistance syndrome prospective study, Diabetes 53 (2004) 1150–1157.
[14] B.B. Duncan, M.I. Schmidt, J.S. Pankow, H. Bang, D. Couper, C.M. Ballantyne, R.C. Hoogeveen, G. Heiss, Adiponectin and the development of type 2 diabetes the atherosclerosis risk in communities study, Diabetes 53 (2004) 2473–2478.
[15] A.S. Lihn, T. Østergård, B. Nyholm, S.B. Pedersen, B. Richelsen, O. Schmitz, Adiponectin expression in adipose tissue is reduced in first-degree relatives of type 2 diabetic patients, Am. J. Physiol. Endocrinol. Metab. 284 (2003) E443–E448.
[16] D.S. Lindsay, T. Funahashi, R.L. Hanson, Y. Matsuzawa, S. Tanaka, P.A. Tataranni, W.C. Knowler, J. Krakoff, Adiponectin and development of type 2 diabetes in the Pima Indian population, Lancet 360 (2002) 57–58.
[17] B. Vozarova, N. Stefan, R.S. Lindsay, J. Krakoff, W.C. Knowler, T. Funahashi, Y. Matsuzawa, M. Stumvoll, C. Weyer, P.A. Tataranni, Low plasma adiponectin concentrations do not predict weight gain in humans, Diabetes 51 (2002) 2964–2967.
[18] W. Yang, W. Lee, T. Funahashi, S. Tanaka, Y. Matsuzawa, C. Chao, C. Chen, T. Tai, L. Chuang, Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin, J. Clin. Endocrinol. Metab. 86 (2001) 3815–3819.
[19] K. Hotta, T. Funahashi, Y. Arita, M. Takahashi, M. Matsuda, Y. Okamoto, H. Iwahashi, H. Kuriyama, N. Ouchi, K. Maeda, M. Nishida, S. Kihara, N. Sakai, T. Nakajima, K. Hasegawa, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Hanafusa, Y. Matsuzawa, Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 1595.
[20] K. Esposito, A. Pontillo, C.D. Palo, G. Giugliano, M. Masella, R. Marfella, D. Giugliano, Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women. A randomized trial, JAMA 289 (2003) 1799–1804.
[21] K. Harada, W.J. Shen, S. Patel, V. Natu, J. Wang, J. Osuga, S. Ishibashi, F.B. Kraemer, Resistance to high-fat diet-induced obesity and altered expression of adipose-specific genes in HSL-deficient mice, Am. J. Physiol. Endocrinol. Metab. 285 (2003) E1182–E1195.
[22] H.C. Chen, D.R. Jensen, H.M. Myers, R.H. Eckel, R.V. Farese Jr., Obesity resistance and enhanced glucose metabolismin mice transplanted with white adipose tissue lacking acyl CoA:diacylglycerol acyltransferase 1, J. Clin. Invest. 111 (2003) 1715–1722.
[23] S. Blüher, M. Ziotopoulou, J.W. Bullen Jr., S.J. Moschos, L. Ungsunan, E. Kokkotou, E. Maratos-Flier, C.S Mantzoros, Responsiveness to peripherally administered melanocortins in lean and obese mice, Diabetes 53 (2004) 82–90.
[24] L. Ma, S. Mao, K.L. Taylor, T. Kanjanabuch, Y. Guan, Y. Zhang, N.J. Brown, L.L. Swift, O.P. McGuinness, D.H. Wasserman, D.E. Vaughan, A.B. Fogo, Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1, Diabetes 53 (2004) 336–346.
[25] E.E. Kershaw, N.M. Morton, H. Dhillon, L. Ramage, J.R. Seckl, J.S. Flier, Adipocyte-specific glucocorticoid inactivation protects against diet-induced obesity, Diabetes 54 (2005) 1023–1031.
[26] B. Yang, P. Lin, K.M. Carrick, J.A. McNulty, L.G. Clifton, D.A. Winegar, J.C. Strum, S.A. Stimpson, G.L. Pahel, PPARγ agonists diminish serum VEGF elevation in diet-induced insulin resistant SD rats and ZDF rats, Biochem. Biophys. Res. Commun. 334 (2005) 176–182.
[27] K. Hotta, T. Funahashi, N.L. Bodkin, H.K. Ortmeyer, Y. Arita, B.C. Hansen, Y. Matsuzawa, 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 (2001) 1126–1133.
[28] T.P. Combs, J.A. Wagner, J. Berger, T. Doebber, W.J. Wang, B.B. Zhang, M. Tanen, A.H. Berg, S. O'Rahilly, D.B. Savage, K. Chatterjee, S. Weiss, P.J. Larson, K.M. Gottesdiener, B.J. Gertz, M.J. Charron, P.E. Scherer, D.E. Moller, Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization, Endocrinology 143 (2002) 998–1007.
[29] W.S. Yang, C.Y. Jeng, T.J. Wu, S. Tanaka, T. Funahashi, Y. Matsuzawa, J.P. Wang, C.L. Chen, T.Y. Tai, L.M. Chuang, Synthetic peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients, Diabetes Care 25 (2002) 376–380.
[30] American Diabetes Association, Physical activity/exercise and diabetes, Diabetes Care. 27 (2004) S58–S62.
[31] E. Ravussin, S.R. Smith, Increased fat intake, impaired fat oxidation, and failure of fat cell proliferation result in ectopic fat storage, insulin resistance, and type 2 diabetes mellitus, Ann. NY Acad. Sci. 967 (2002) 363–378.

[32] W.W. Winder, Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle, J. Appl. Physiol. 91 (2001) 1017–1028.
[33] B. Yang, L.G. Clifton, J.A. McNulty, L. Chen, K.K. Brown, P.G. Baer, Effects of a PPARgamma agonist, GI262570, on renal filtration fraction and nitric oxide level in conscious rats, J. Cardiovasc. Pharmacol. 42 (2003) 436–441.
[34] T.M. Willson, M.H. Lambert, S.A. Kliewer, Peroxisome proliferation-activated receptor gamma and metabolic disease, Annu. Rev. Biochem. 70 (2001) 341–367.
[35] J.M. Chirgwin, A.E. Przybyla, R.J. MacDonald, W.J. Rutter, Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease, Biochemistry 18 (1979) 5294–5299.
[36] S. Bustin, Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays, J. Mol. Endocrinol. 25 (2000) 169–193.
[37] C. Weyer, T. Funahashi, S. Tanaka, K. Hotta, Y. Matsuzawa, R.E. Pratley, P.A. Tataranni, Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia, J. Clin. Endocrinol. Metab. 86 (2001) 1930–1935.
[38] Y. Yamamoto, H. Hirose, I. Saito, M. Tomita, M. Taniyama, K. Matsubara, Y. Okazaki, T. Ishii, K. Nishikai, T. Saruta, Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population, Clin. Sci. 103 (2002) 137–142.
[39] E.K. Naderali, D. Estadella, M. Rocha, L.C. Pickavance, S. Fatani, R.G. Denis, G. Williams, A fat-enriched, glucose-enriched diet markedly attenuates adiponectin mRNA levels in rat epididymal adipose tissue, Clin. Sci. 105 (2003) 403–408.
[40] T. Yamauchi, J. Kamon, Y. Ito, A. Tsuchida, T. Yokomizo, S. Kita, T. Sugiyama, M. Miyagishi, K. Hara, M. Tsunoda, K. Murakami, T. Ohteki, S. Uchida, S. Takekawa, H. Waki, N.H. Tsuno, Y. Shibata, Y. Terauchi, P. Froguel, K. Tobe, S. Koyasu, K. Taira, T. Kitamura, T. Shimizu, R. Nagai, T. Kadowaki, Cloning of adiponectin receptors that mediate antidiabetic metabolic effects, Nature 423 (2003) 762–769.
[41] T.S. Tsao, H.E. Murrey, C. Hug, D.H. Lee, H.F. Lodish, Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30), J. Biol. Chem. 277 (2002) 29359–29362.
[42] 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.
[43] f.f.M. Fisher, M.E. Trujillo, W. Hanif, A.H. Barnett, P.G. McTernan, P.E. Scherer, S. Kumar, Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males, Diabetologia 48 (2005) 1084–1087.
[44] U.B. Pajvani, M. Hawkins, T.P. Combs, M.W. Rajala, T. Doebber, J.P. Berger, J.A. Wagner, M. Wu, A. Knopps, A.H. Xiang, K.M. Utzschneider, S.E. Kahn, J.M. Olefsky, T.A. Buchanan, P.E. Scherer, Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity, J. Biol. Chem. 279 (2004) 12152–12162.