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Tissue-Specific Effect of Dietary Cysteamine on Expression of Adiponectin Receptors in Rats

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Adiponectin is synthesized by adipocytes and affects glucose and lipid metabolism by binding to its receptors, AdipoR1 and AdipoR2. Cysteamine, a naturally existing intermediate metabolite of sulfur amino acid, has been reported to modulate metabolism and growth in various species of animals; however, whether the action of cysteamine involves adiponectin and its receptors is unknown. The objective of the present study was therefore to investigate the effect of dietary cysteamine on the expression of AdipoR1/R2 in different tissues, in association with the alterations in endocrine and metabolic status. Rats were fed either of the diets supplemented with 0 or 700 mg/kg cysteamine feed additive (containing 30% of cysteamine hydrochloride) for 4 weeks, and the expression of adiponectin and its receptors in adipose tissue, AdipoR1 and AdipoR2 in liver, gastrocnemius, and soleus muscle was determined, in association with the growth performance and serum concentrations of hormones and metabolites. A temporal trend of increase in growth rate and the ratio of feed consumption relative to body weight gain was observed in the second week of cysteamine supplementation. Serum concentrations of insulin and TNF-α increased, while serum levels of triglycerides, FFA, and total cholesterol decreased significantly 4 weeks after cysteamine treatment. Leptin and GH remained unaffected. Cysteamine supplementation increased mRNA expression of AdipoR1 in adipose tissue, gastrocnemius, and soleus muscle as well as that of AdipoR2 in soleus muscle and adipose tissue. Nevertheless, hepatic expression of AdipoR1 and AdipoR2 was not influenced. Despite a numeric increase, no significant alteration in adiponectin mRNA expression in adipose tissue was observed. In conclusion, dietary supplementation of cysteamine modulates the endocrine and metabolic status of rats, which may involve the tissue-specific responses of adiponectin receptors at the level of mRNA transcription.

KEYWORDS: Adiponectin; adiponectin receptors; cysteamine; rat

INTRODUCTION

Adiponectin is an adipokine which is exclusively secreted from the adipose tissue to exert profound effects on glucose utilization and lipid metabolism (*1*–*3*). Adiponectin exerts its action by binding to two specific receptors, AdipoR1 and AdipoR2. AdipoRl was found to be ubiquitously expressed, and the most abundant expression was found in skeletal muscle, whereas AdipoR2 is predominately expressed in liver (*4*). The expression of different types of adiponectin receptor was found to be regulated in a tissue- and species-specific manner under different endocrine and metabolic status. Growth hormone (GH) was reported to induce AdipoR2, but not AdipoR1 mRNA expression in 3T3-L1 adipocytes (*5*), whereas in the cultured human adipose tissue, GH decreased AdipoR2 expression (*6*). Insulin seems to negatively regulate AdipoR1/R2 mRNA levels in skeletal muscle (*7*), and the reduced expression of AdipoR1/R2 under the state of obesity has been implicated to be responsible for the development of insulin resistance and type 2 diabetes (*7*, *8*). Therefore, it is suggested that strategies to increase AdipoR1/R2 may provide a novel treatment for insulin resistance and type 2 diabetes (*3*).

Skeletal muscle is a primary site of glucose disposal and fatty acid oxidation and plays an important role in the regulation of metabolic homeostasis. Different types of muscle fiber have different oxidation capacity, and it is reasonable to presume that the expression and regulation of the adiponectin receptors are

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Table 1. Nutritional Composition of the Basal Diet[a]

nutritional composition	content
water content (%)	9.7
crude protein (%)	20.5
crude fat (%)	4.62
crude ash (%)	6.2
crude fiber (%)	4.35
nitrogen-free extract (%)	52.5
calcium (%)	1.23
phosphorus (%)	0.91
calcium:phosphorus	1.35
lysine (%)	1.3
methionine + cysteine (%)	0.68
gross energy (kcal/kg)	345

[a] The vitamins and trace minerals were included in the premix according to the National Standard for rats (GB 14924.3-2001).

different in muscles differ in myofiber type composition. However, no data describing such difference are available up to now.

Cysteamine is known to modulate the endocrine and metabolic status of animals via deleting endogenous somatostatin (*9–11*). Various hormones, including growth hormone (*9, 12, 13*), insulin (*9, 13–15*), thyroid hormones (*16*), and IGF-I (*17*), are observed to be affected by cysteamine treatment. It has been demonstrated that cysteamine improves growth performance, inhibits fat deposition, and promotes muscle accretion rate (*17–19*). Questions arise whether adiponectin and its receptors are involved in the metabolic effect of cysteamine and in what manner the expression of adiponectin and its receptors are modulated by cysteamine.

Therefore, the objective of the present study was to investigate the effect of dietary cysteamine on the expression of AdipoR1/R2 in liver, fat, and two different types of muscle with different oxidation capacities, gastrocnemius and soleus, in association with the alterations in hormone and metabolic status of rats.

MATERIALS AND METHODS

Animals and Experimental Design. Twenty male Wistar rats initially weighing 190–210 g were purchased from Shanghai Center for Experimental Animals, Chinese Academy of Science. Rats were maintained under controlled temperature (22 ± 1 °C), humidity, and air flow conditions, with a fixed 12L:12D lighting regimen with lights on from 0700 to 1900. All rats were provided ad libitum a standard basal diet with the nutritional composition shown in **Table 1** during 1 week of acclimation. Then the rats were randomly divided into two groups: the cysteamine-treated group ($n = 10$) received a diet supplemented with a commercial cysteamine feed additive (supplied by Shanghai Walcom Bio-Chem Co., Ltd., containing 30% cysteamine hydrochloride with starch and dextrine as carriers for stabilization) at the level of 700 mg/kg, which is recommended for improving farm animal growth, while the control group ($n = 10$) received the basal diet supplemented with the same amount of starch and dextrine. The experiment lasted for 4 weeks. The body weight and food consumption were recorded every week during the experimental period. At the end of the dietary treatment, food was removed at the end of the dark period (0700), and rats were anesthetized with intraperitoneal injection of pentobarbital sodium (60 mg/kg body weight). Blood samples were obtained from the jugular vein, and serum was stored at −20 °C until measurements. Liver, gastrocnemius muscle, soleus muscle, and retroperitoneal adipose tissue were removed and immediately minced, snap frozen in liquid nitrogen, and stored at −80 °C. The experiment was undertaken following the guidelines of the regional animal ethics committee.

Hormones and Metabolites Measurements. Serum GH, insulin, TNF-α, and leptin concentrations were measured with respective commercial RIA kits purchased from Beijing North Institute of Biotechnology, Beijing, China. The detection limits for GH, TNF-α, leptin, and insulin were 0.5 ng/mL, 0.3 ng/mL, 0.5 ng/mL, and 1.0 μIU/mL, respectively. The intra- and interassay coefficients of variation were 6.5% and 9.5% for GH, 5% and 8% for TNF-α, 5% and 10% for letptin, and 8% and 10% for insulin.

Serum triglycerides, FFA, and total cholesterol concentrations were determined with respective commercial kits (Nanjing Jiancheng Biochemical Reagent Co., Nanjing, China).

RNA Isolation and RT-PCR. Total cellular RNA was extracted by using TRNzol reagent (Tiangen Biotech Co., Ltd., Beijing, China). RNA concentration was then quantified by measuring the absorbance at 260 nm in a photometer (Eppendorf Biophotometer). Ratios of absorption (260/280 nm) of all preparations were between 1.9 and 2.0. 2 μg of total RNA was reverse transcribed by incubation at 37 °C for 1 h in a 25 μL mixture consisting of 100 U M-MLV reverse transcriptase (Promega, Madison, WI), 8 U RNase inhibitor (Promega, Madison, WI), 21 μmol/L random primers (6 bp), 50 mmol/L Tris-HCl (pH 8.3), 3 mmol/L $MgCl_2$, 75 mmol/L KCl, 10 mmol/L DDT, and 0.8 mmol/L each dNTP (Promega, Madison, WI). The reaction was terminated by heating at 95 °C for 5 min and quickly cooling on ice.

The mRNA expression of adiponectin in adipose tissue, AdipoR1/R2 in liver, fat, gastrocnemius muscle, and soleus muscle was quantified relative to 18S rRNA, using the Quantum RNA 18S Internal Standards kit (catalogue no. 1716, Ambion Inc., Austin, TX), containing primers and competitors, for normalizing variations in pipetting and amplification. 2 μL of RT reaction mix was used for PCR in a final volume of 25 μL containing 0.5 U Taq DNA polymerase (Promega, USA), 5 mmol/L Tris-HCl (pH 9.0), 10 mmol/L NaCl, 0.1 mmol/L DDT, 0.01 mmol/L EDTA, 5% (w/v) glycerol, 0.1% (w/v) Triton X-100, 0.2 mmol/L each dNTP, 1.6 mmol/L $MgCl_2$, and 0.4 μmol/L of each primer pair specific for adiponectin, AdipoR1, and AdipoR2, respectively, together with 0.4 μmol/L 18S rRNA primers and competitors in proportions optimized for each target gene (1:1 for AdipoR1, 7:3 for AdipoR2, and 4:1 for adiponectin). Each target gene was coamplified with 18S rRNA in the same reaction. The primers for target genes were designed using Primer Premier 5.0 and were synthesized by Yinjun Biotech. Ltd., China. The nucleotide sequences of these primers and the PCR conditions set for respective genes are shown in **Table 2**. The PCR products from each reaction were sent to Haojia Biotech. Ltd. China for sequencing to verify the specificity. The reported sequences matched exactly to that published in GenBank. Different controls were set to monitor the possible contaminations of genomic DNA and environmental DNA both at the stage of RT and PCR. The pooled samples made by mixing equal quantity of total cDNA from all samples were used for optimizing the PCR conditions and normalizing the intra-assay variations. The cycles of PCR were determined to ensure that the amplifications were terminated within the linear range for quantitation. All samples were included in the same run of RT-PCR and repeated at least for three times. Both RT and PCR were performed in a Gene Amp PCR System 9600 (Perkin-Elmer, Waltham, MA).

Quantitation of PCR Products. An aliquot (5–10 μL) of PCR products was analyzed by electrophoresis on 1.4% agarose gels. The gels were stained with GoldView and photographed with Gel Image System (Tanon 2100, Shanghai, China). The net intensities of individual bands were measured using Gel Image System software. The ratios of net intensity of target genes to 18S rRNA were used to represent the relative levels of target gene expression. The results were expressed as mean ± SEM.

Statistical Analyses. The effects of dietary cysteamine on the growth, serum hormones and metabolites, and mRNA expression of adiponectin in adipose tissue were analyzed using one-way ANOVA of SPSS13.0 for Windows. The expression of adiponectin receptors were analyzed using the General Linear Model of SPSS13.0 for Windows. Least-squares means were evaluated for significant differences in a general linear model for all dependent variables associated with the dosage of cysteamine as fixed factor and different tissues as random factor. The one-way ANOVA test was used again to compare the differences from the control group when the F-test was significant ($P < 0.05$).

Table 2. Nucleotide Sequences of Specific Primers and PCR Conditions

target genes	PCR products (bp)	GenBank accession	primer sequences	PCR conditions
AdipoR1	443	NM-207587	5′-tggactattcagggattgc-3′ 3′-aaagcaggtgaagataccc-5′	95 °C, 40 s, 54 °C, 40 s, 72 °C, 60 s 32 cycles
AdipoR2	168	NM-001037979	5′-gccaccatagggcagatag- 3′ 3′-ccaacgaccacggaaacaa-5′	95 °C, 40 s, 54 °C, 40 s, 72 °C, 60 s 30 cycles
Adiponectin	133	BC092565	5′-tcttggtcctaagggtgac- 3′ 3′-agtgagtcgtaagtcgcat-5′	95 °C, 40 s, 57 °C, 40 s, 72 °C, 60 s 28 cycles

Table 3. Body Weight Gain and Feed Conversion Ratio (FCR) in Control and Cysteamine-Treated Rats[a]

	control		cysteamine	
age (week)	FCR	body weight (g)	body weight (g)	FCR
0		213.18 ± 3.61	213.87 ± 2.56	
1	5.40	263.67 ± 4.30	263.00 ± 2.37	5.38
2	6.13	300.17 ± 3.26	306.83 ± 1.45	3.93
3	5.61	341.17 ± 5.04	345.83 ± 1.76	5.22
4	6.41	384.50 ± 5.60	390.00 ± 2.52	6.05

[a] Values are expressed as means and SEM. Ten animals were included in each group.

Table 4. Serum Levels of Hormones and Metabolites in Control and Cysteamine-Treated Rats[a]

	control	cysteamine
GH (ng/mL)	6.63 ± 0.33 a	6.69 ± 0.21 a
insulin (μIU/mL)	20.52 ± 4.03 a	33.51 ± 4.66 b
TNF-α (ng/mL)	1.69 ± 0.02 a	1.97 ± 0.07 b
leptin (ng/mL)	5.56 ± 0.23 a	5.88 ± 0.22 a
TG (mmol/L)	2.10 ± 0.14 a	1.26 ± 0.04 b
FFA (μmol/L)	3418.4 ± 198.5 a	2658.0 ± 134.7 b
cholesterol (mmol/L)	1.75 ± 0.06 a	1.51 ± 0.04 b

[a] Values are expressed as means and SEM. Ten animals were included in each group. Values in each row that do not share the same letter are significantly different ($P < 0.05$, between control and cysteamine group).

RESULTS

Growth Performance. As shown in **Table 3**, the body weight and feed conversion ratio (FCR) in cysteamine-treated group were slightly increased compared with that in control group. A trend of increase ($P = 0.1$) in body weight was observed in the second week of dietary cysteamine supplementation. However, such difference in body weight diminished in subsequent weeks.

Serum Concentrations of Hormones and Metabolites. Four weeks after cysteamine treatment, serum concentrations of insulin and TNF-α significantly increased ($P = 0.02$ and $P = 0.05$), while serum levels of triglycerides, FFA, and total cholesterol decreased significantly ($P = 0.00$, $P = 0.01$, and $P = 0.00$, respectively); leptin and GH remained unaffected ($P = 0.33$ and $P = 0.98$), as shown in **Table 4**.

Expression of AdipoR1, AdipoR2, and Adiponectin mRNA in Different Tissues. Semiquantitative RT-PCR revealed tissue-specific pattern as well as the tissue-specific effect of cysteamine on AdipoR1/R2 expression.

As shown in **Figure 1**, the highest abundance of AdipoR1 mRNA was shown in gastrocnemius muscle, followed by soleus muscle, liver, and abdominal fat. Dietary supplementation of cysteamine for 4 weeks significantly increased mRNA expression of AdipoR1 in adipose tissue, gastrocnemius, and soleus muscle ($P = 0.01$, $P = 0.04$, and $P = 0.01$, respectively). Nevertheless, hepatic expression of AdipoR1 was not affected ($P = 0.64$).

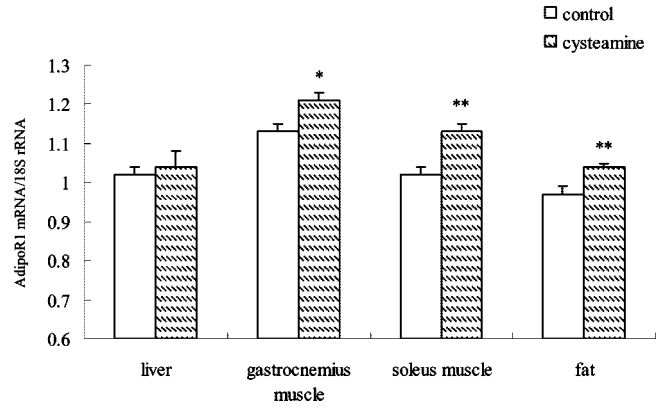

Figure 1. AdipoR1 mRNA abundances in different tissues analyzed by RT-PCR expressed as the ratio of AdipoR1 mRNA to 18S rRNA. Values are mean ± SEM ($n = 10$). * $p < 0.05$, ** $p < 0.01$, compared with the control group.

AdipoR2 was expressed in all tissues analyzed, and the highest expression was observed in liver and fat followed by soleus muscle and gastrocnemius muscle. Dietary supplementation of cysteamine for 4 weeks augmented AdipoR2 mRNA expression in adipose tissue and soleus muscle ($P = 0.05$ and $P = 0.02$), but not in liver and gastrocnemius muscle ($P = 0.25$ and $P = 0.73$) (**Figure 2**).

Despite a numeric increase, no significant alteration was observed in adiponectin mRNA expression in adipose tissue 4 weeks after cysteamine treatment (**Figure 3**).

DISCUSSION

The present study demonstrated the tissue-specific responses of AdipoR1/R2 mRNA to dietary supplementation of cysteamine in rats, in association with the alterations in growth performance, as well as the endocrine and metabolic status of rats.

The growth-promoting effect of cysteamine has been demonstrated in fish and chicken (*17*, *19*, *20*), and the efficacy of cysteamine seems to depend on dose, timing and route of administration, duration of use, species, and the metabolic and physiological state of the animal. No data are available describing the growth-promoting effect of CSH in mammals to our knowledge. A temporal improvement in growth rate was observed in the present study 2 weeks after the dietary treatment of cysteamine at the dosage of 700 mg/kg cysteamine feed additive (containing ∼210 mg/kg CSH) in rats. Since the

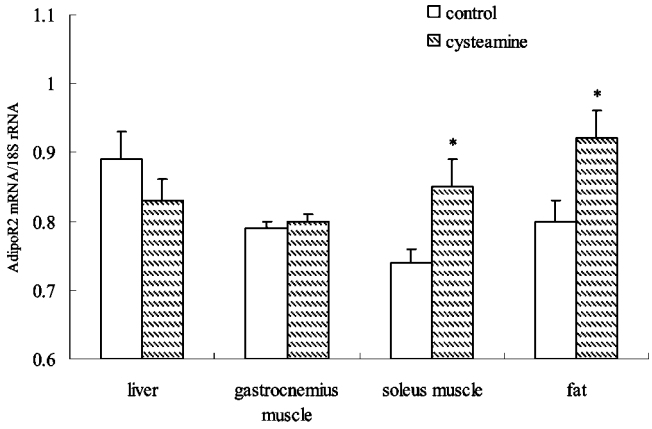

Figure 2. AdipoR2 mRNA abundances in different tissues analyzed by RT-PCR expressed as the ratio of AdipoR2 mRNA to 18S rRNA. Values are mean ± SEM ($n = 10$). * $p < 0.05$ compared with the control group.

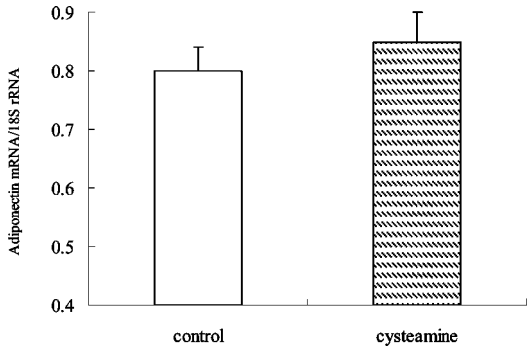

Figure 3. Adiponectin mRNA abundances in abdominal fat analyzed by RT-PCR expressed as the ratio of adiponectin mRNA to 18S rRNA. Values are mean ± SEM ($n = 10$).

commercial cysteamine feed additive used in the present study is microencapsulated, the stability of CSH supplemented in the feed is ensured. The effective dose may thus not be comparable with other studies using other CSH preparations. The optimal dose and the timing of administration for growth-promoting effect of the commercial cysteamine feed additive in rats are to be determined.

The growth-promoting action of cysteamine was suggested to be mediated by its effect on depletion of endogenous somatostatin (*10*, *11*, *16*, *18*). Somatostatin is broadly secreted in both the central nervous system and various peripheral tissues and participates in the regulation of many physiological functions including gastrointestinal functions, metabolism, and growth. Therefore, as expected, cysteamine demonstrated remarkable effects on the endocrine system of both human and animals in different manners. The effect of cysteamine hydrochloride on depletion of somatostatin is demonstrated at the dose of 30 mg/kg BW (BW = body weight) within 2–4 h of a single injection, which was found to be largely reversible within 1 week (*21*, *22*). Cysteamine was shown to increase temporarily the secretion of GH in sheep, rats, and chickens in a dose-dependent manner (*9*, *12–14*, *23*). Intragastric administration of cysteamine at 50 mg/kg BW in sheep augmented the mean plasma growth hormone concentration, with the greatest response occurring on day 3. Baseline growth hormone concentration were elevated in wethers dosed with 50 mg cysteamine/kg BW on day 3, whereas wethers dosed with 100 mg of cysteamine hydrochloride/kg BW had lower baseline GH concentration on day 0 (*9*, *13*). Moreover, different doses of cysteamine can influence gastric juice secretion, gastric emptying, and the gastric blood flow (*24*, *25*). In the present study, dietary supplementation of cysteamine for 4 weeks did not change the serum GH level. This may be resulted from the dosage as well as the timing and/or the method of blood sampling. The dosage of cysteamine applied in the present study may not be sufficient to influence the GH secretion, or the effect escaped detection because of the mistiming of the sampling. Since GH secretion is pulsatile in nature, it could be that cysteamine affected the pulse amplitude, and this influence could not be reflected by the mean value of serum GH, as determined in the present study using single sampling.

Despite unaltered serum GH levels, cysteamine supplementation demonstrated significant influence on hormones and metabolites involved in glucose and lipid metabolism (*14*, *18*, *26*). Serum insulin levels increased significantly 4 weeks after cysteamine supplementation in the present study, being consistent with the previous findings (*9*, *13–15*). Moreover, significantly elevated TNF-α levels implied the influence of cysteamine in energy homeostasis and lipid metabolism. Higher levels of TNF-α mRNA and protein are associated with obesity in humans and pigs (*27–29*). Moreover, TNF-α was the first adipocytokine proposed to represent a molecular link between obesity and insulin resistance (*30*). The increased serum levels of TNF-α, combined with decreased serum levels of triglycerides, FFA, and total cholesterol, may probably lead to a presumption of increased fat deposition. Plasma leptin levels that reflect the sum of all peripheral leptin production correlate closely with the body fat mass and adipocyte cell size in both lean and obese mice (*31*); to our surprise, in the present study, the leptin level and the body fat content were not altered in the cysteamine-treated group (data not shown). The increased serum insulin levels may induced by the direct effect of cysteamine on pancreas to attenuate the inhibitory effect of somatostatin on insulin secretion (*15*, *25*) and may facilitate the glucose utilization in peripheral tissues, while the decreased serum level of TG and FFA may indicate the enhanced uptake and utilization of TG and FFA in liver and/or muscle. In the present study we failed to observe a significant effect of cysteamine on adipose adiponectin mRNA expression; however, one cannot rule out the possibility that cysteamine may affect the serum level of adiponectin, as the serum adiponectin level is not always consistent with the mRNA expression (*32*).

Adiponectin is shown to stimulate the glucose uptake and fatty acid oxidation through the mediation of adiponectin receptors (*3*). The tissue distribution pattern of adiponectin receptors observed in the present study confirms the previous finding that AdipoR1 is mostly distributed in skeletal muscle, while AdipoR2 is mostly distributed in the liver (*4*). Adipose tissue was shown to express abundant mRNAs for both AdipoR1 and AdipoR2, indicating autocrine and/or paracrine functions of adiponectin on adipocytes, apart from its known endocrine functions. In addition, soleus muscle was shown to express less AdipoR1 mRNA compared with gastrocnemius muscle. Soleus muscle has higher oxidation capacity, and it is possible that adiponection affects the glucose and lipid metabolism in a muscle type specific fashion.

Dietary supplementation of cysteamine significantly increased AdipoR1 mRNA expression in gastrocnemius and soleus muscles as well as AdipoR2 mRNA expression in soleus muscle and adipose tissue, suggesting a tissue-specific response of adiponectin receptors to cysteamine treatment. Some hormones and cytokines, including GH, insulin, prolactin, TNF-α, IFN-γ, and IL-6, have been suggested to influence the expression

of adiponectin receptors. However, the data available up to date are quite confusing. Fasshauer et al. found that growth hormone induced AdipoR2, but not AdipoR1 mRNA in 3T3-L1 adipocytes (*5*), while Nisson et al. reported that GH decreased AdipoR2 expression in the cultured human adipose tissue (*6*). Staiger et al. found that AdipoR1 mRNA expression in human myotubes was positively correlated with insulin concentration (*33*), while Tsuchida et al. showed that insulin deficiency induced by streptozotocin increased and insulin replenishment reduced the expression of AdipoR1/R2 in skeletal muscles of mice in vivo (*7*). Different experimental design and model may contribute to the contradictory results. The cysteamine-induced alteration in the expression of adiponection receptors may possibly be induced by changes of endocrine status, as observed in the present study. The augmented expression of AdipoR1 in skeletal muscles and fat and AdipoR2 in soleus muscle and fat may indicate enhanced glucose utilization in skeletal muscle and the lipid mobilization in adipose tissue.

In conclusion, the present study provides the evidence that dietary supplementation of cysteamine modulates the endocrine and metabolic status of rats, which may involve the tissue-specific responses of adiponectin receptors at the level of mRNA transcription. The mechanisms underlying this effect and the potential application of cysteamine in regulating the metabolic homeostasis await further investigation.

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