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Metformin Enhances Leptin Sensitivity in Aged Rats

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To evaluate whether metformin restores leptin sensitivity in aged rats with leptin resistance, we measured leptin sensitivity in aged (2 year old) and adult (5 month old) rats after 4 weeks of treatment with metformin (300 mg/kg/D, mixing in drinking water), by measuring food intake, body weight and visceral fat losing effects. Leptin (15 μg/D) was administered by intracerobroventricular (i.c.v.) infusion through osmotic minipump for 1 week. Metformin treatment decreased body weight and daily food intake in both adult and aged rats compared with their control rats, however, these effects were more prominent in aged rats than in adult rats. Anorexic and fat losing responses following i.c.v. leptin were attenuated in aged rats compared to adult rats. However, these responses of aged rats to leptin were restored by metformin treatment. Moreover, serum concentration of leptin in aged rats was significantly decreased by combined treatment with metformin and leptin. These results suggest that metformin enhances leptin sensitivity in aged rat model, and that combination therapy with metformin and leptin would be helpful for treatment of aging-associated obesity.

Key Words: Leptin resistance, Food intake, Aged rats, Visceral fat

INTRODUCTION

The metabolic characteristics of aged subjects are central obesity, hyperlipidemia, insulin resistance, and leptin resistance, which are associated with metabolic syndrome (van den Brandt et al, 2002; Cankurtaran et al, 2006). Moreover, in an animal study, obesity, a major component of metabolic syndrome, is progressively increased parallel with aging process in spite of the elevated leptin (Scarpace et al, 2000).

Leptin, an adipocyte derived hormone, contributes to body weight homeostasis through regulation of both food intake and energy expenditure (Halaas et al, 1995). Nevertheless, leptin is not widely being used in the clinical field, because obesity and aging are accompanied by leptin resistance and they respond poorly to exogenous leptin that ordinarily promotes reduction of fat mass and body weight in lean subjects (Considine et al, 1996; Shek and Scarpace, 2000). Thus, enhancement of leptin sensitivity could be an excellent treatment modality for obesity.

Metformin, an oral biguanide insulin-sensitizing agent, inhibits hepatic glucose production, enhances the effects of insulin on glucose uptake in skeletal muscles and adipocytes, and decreases intestinal absorption of glucose (Hundal et al, 1992; Klip et al, 1992; Velazquez et al, 1997; Nestler et al, 1998; Wiernsperger and Bailey, 1999). It is also well known that metformin decreases body weight, which is the reason of why metformin has been prescribed to treat obese type 2 diabetic patients. Metformin also decreases leptin concentration in morbidly obese subjects (Glueck et al, 2001; Kay et al, 2001), healthy men with normal weight (Fruehwald-Schultes et al, 2002), and even in obese polycystic ovary syndrome patients (Morin-Papunen et al, 1998). Moreover, we recently reported that metformin increases leptin sensitivity (Kim et al, 2004). Thus, metformin could be considered as a strong candidate for restoring leptin sensitivity in aged rats with leptin resistance. In this study, we measured the effect of metformin on leptin sensitivity in aged rats.

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METHODS

Animal care

Two years old Sprague-Dawley male rats were used as aged rats in this study (Scarpace et al, 2000), while five months old male rats were used as adult rat. The experimental rats were purchased from Daehan Experimental Animal Center (Seoul, Korea). The rats were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals of the Yeungnam Medical Center. Rats were individually housed under a 12 : 12 h light : dark cycle (07 : 00 to 19 : 00 h).

Experimental design

To study whether metformin can enhance leptin sensitivity in the leptin resistant rat model, aged and adult rats were treated with metformin for 4 weeks and then compared with untreated control rats. These control and

ABBREVIATIONS: AMPK, AMP-activated protein kinase; PCOS, polycystic ovarian syndrome.

metformin treated aged and adult rats were further divided into Vehicle and Leptin groups (n=6−8 in each group). The cumulative food intake and changes of body weight were measured following intracerebroventricular (i.c.v.) infusion of vehicle or leptin for 7 days. Food intake was measured manually every morning.

Metformin treatment

Metformin (300 mg/kg body weight/day), dissolved in drinking water, was administered orally for 4 weeks. Metformin concentrations in water were readjusted twice a week by measuring daily water intake.

Leptin or vehicle administration

Rats were administered with either vehicle or leptin (15 μg/rat/day) for 7 days by i.c.v. infusion through osmotic minipump (Alzet, CA, USA) according to the method previously described (Kim et al, 2005). Artificial cerebrospinal fluid was used as a vehicle. Rats were anesthetized with xylazine hydrochloride (8 mg/kg body weight, subcutaneously) and ketamine (90 mg/kg body weight, intraperitoneally). After a surgical plane of anesthesia was reached, the animal's head was prepared for surgery, and the animal was placed into a stereotaxic frame. A small incision was made over the midline of the skull to expose land marks of the cranium (bregma and lamda). A brain infusion cannula (Alzet, CA) was stereotaxically placed into the lateral ventricle using the following coordinates, 1.3 mm posterior to bregma, 1.9 mm lateral to the midsagittal suture, and to a depth of 4.0 mm. A small hole was drilled through the skull and a brain infusion kit (Alzet) was inserted through the hole and connected with osmotic minipump settled on the subcutaneous of back area. The cannula was secured to the surface of the skull with jeweler's screw and acrylic dental cement. The infusion rate used was 1 μl/hr. The proper placement of cannula was confirmed through an observation of the hole made by the cannula in the lateral ventricle during tissue harvesting.

Tissue harvesting and preparation

Rats were anesthetized with pentobarbital (85 mg/kg body weight, intraperitoneally). Blood samples were collected through the heart puncture, and serum was harvested by centrifugation of serum separator tubes for 10 min. Serum was quickly frozen with liquid nitrogen and stored at −70°C. Visceral fat mass, including epididymal, retroperitoneal and perirenal fat depots, was excised and weighed.

Measurement of leptin

Serum leptin level was measured using a rat leptin radioimmunoassay kit (Linco Research, MO, USA).

Statistical analysis

Data are presented as mean±SE. Differences between the groups were analyzed by repeatedly measured ANOVA. The differences of visceral fat mass and serum leptin concentration were analyzed by t-test. A value of $P<0.05$ was considered statically significant.

RESULTS

Characteristics of aged rats

Visceral fat mass was increased two-fold in aged rats compared to adult rats, although their body weight was only 25% higher than in adult rats (Figs. 1 and 5). Serum leptin concentration was significantly increased in aged rats compared to adult rats ($p<0.01$) (Fig. 6).

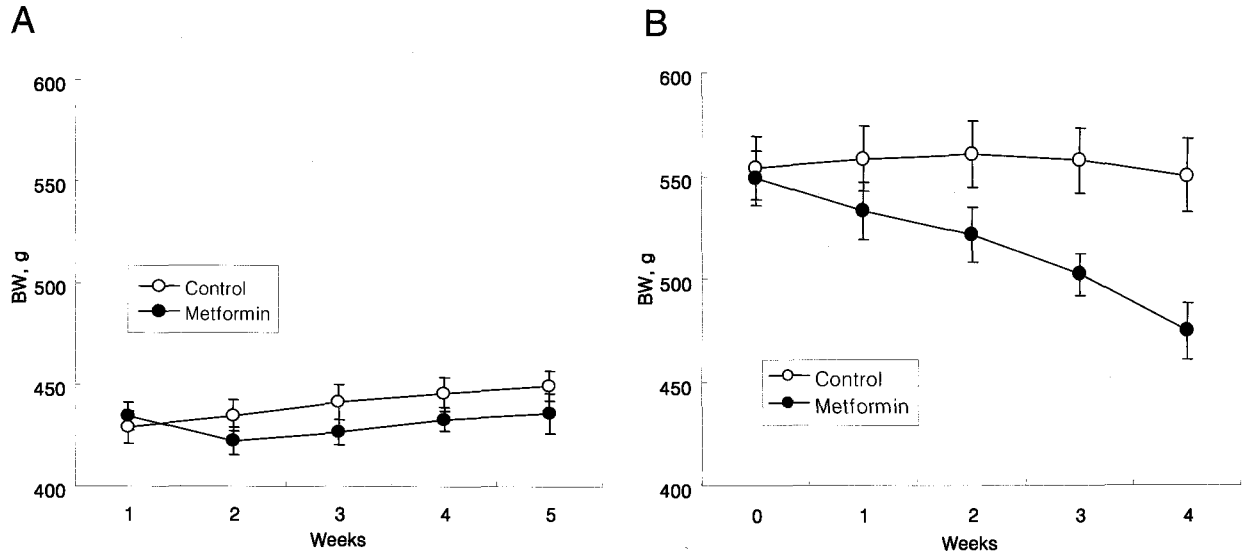

Fig. 1. Effect of metformin treatment for 4 weeks on body weights in adult (A) and aged (B) rats. Values represent mean ± S.E. of 6~8 rats per group. $P<0.01$ and $P<0.0001$ for difference between control and metformin treatment by ANOVA with repeated measures in both adult and aged rats, respectively.

The effect of metformin treatment in aged rats

Metformin treatment decreased caloric intake and body weight in both adult and aged rats. The decreases of caloric intake and body weight, however, were more marked in aged rats than those in adult rats (Figs. 1 and 2).

The effect of i.c.v. infusion of leptin on food intake, body weight and visceral fat mass

Anorexic effect of leptin was determined by changes of daily caloric intake following an i.c.v. leptin infusion. Cumulative food intake was deceased by leptin infusion in adult rats, however, the decrease was attenuated in aged rats, suggesting a development of leptin resistance. However, the anorexic effect of leptin in aged rats was increased by metformin treatment. The pattern of body weight changes following i.c.v. leptin infusion in adult rats was similar to those of cumulative food intake. Body weight was also decreased dramatically by leptin infusion in metformin treated aged rats compared to control rats (Figs. 3 and 4).

Leptin treatment decreased visceral fat mass in adult

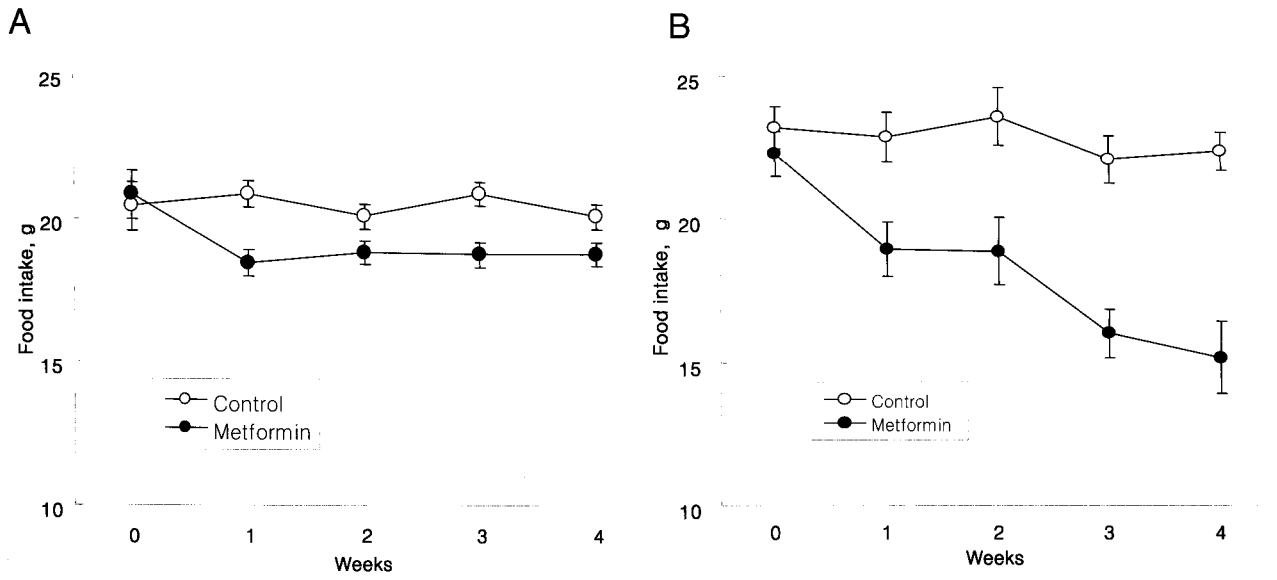

Fig. 2. Effect of metformin treatment for 4 weeks on food intake in adult (A) and aged (B) rats. Values represent mean ± S.E. of 6~8 rats per group. $P<0.01$ and $P<0.0001$ for difference between control and metformin treatment by ANOVA with repeated measures in both adult and aged rats, respectively.

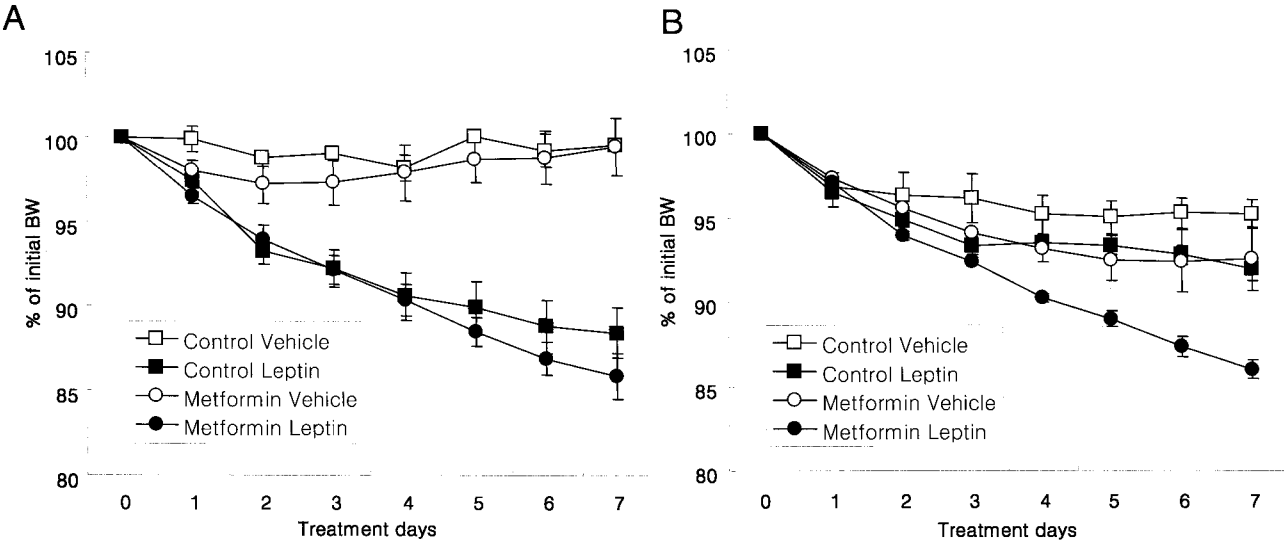

Fig. 3. Effects of intracerebroventricular (i.c.v.) leptin infusion on body weight in adult (A) and aged (B) rats. Leptin (15 μg/day) or vehicle (artificial cerebrospinal fluid) was infused for 7 days. Values are means±S.E. of 6~8 rats per group. $P<0.0001$ for difference between vehicle and leptin by ANOVA with repeated measures in adult and metformin treated aged rats. $P<0.01$ for difference between vehicle and leptin by ANOVA with repeated measures in control aged rats.

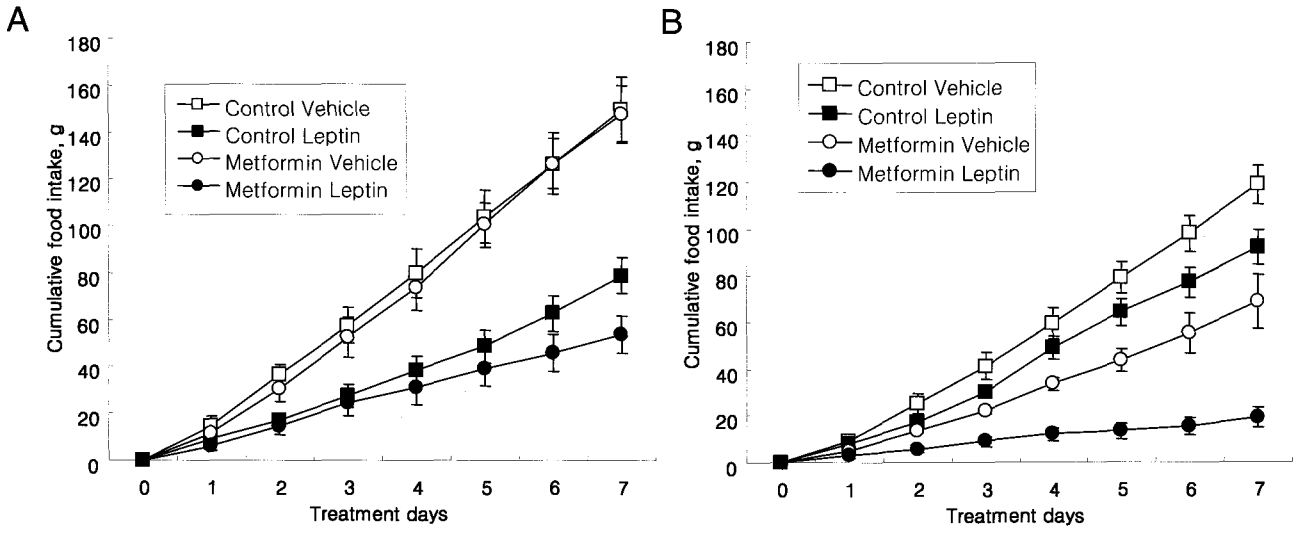

Fig. 4. Effects of intracerebroventricular (i.c.v.) leptin infusion on cumulative food intake in adult (A) and aged (B) rats. Leptin (15 μg/day) or vehicle (artificial cerebrospinal fluid) was infused for 7 days. Values are means±S.E. of 6~8 rats per group. $P<0.0001$ for difference between vehicle and leptin by ANOVA with repeated measures in adult and metformin treated aged rats. $P<0.01$ for difference between vehicle and leptin by ANOVA with repeated measures in control aged rats.

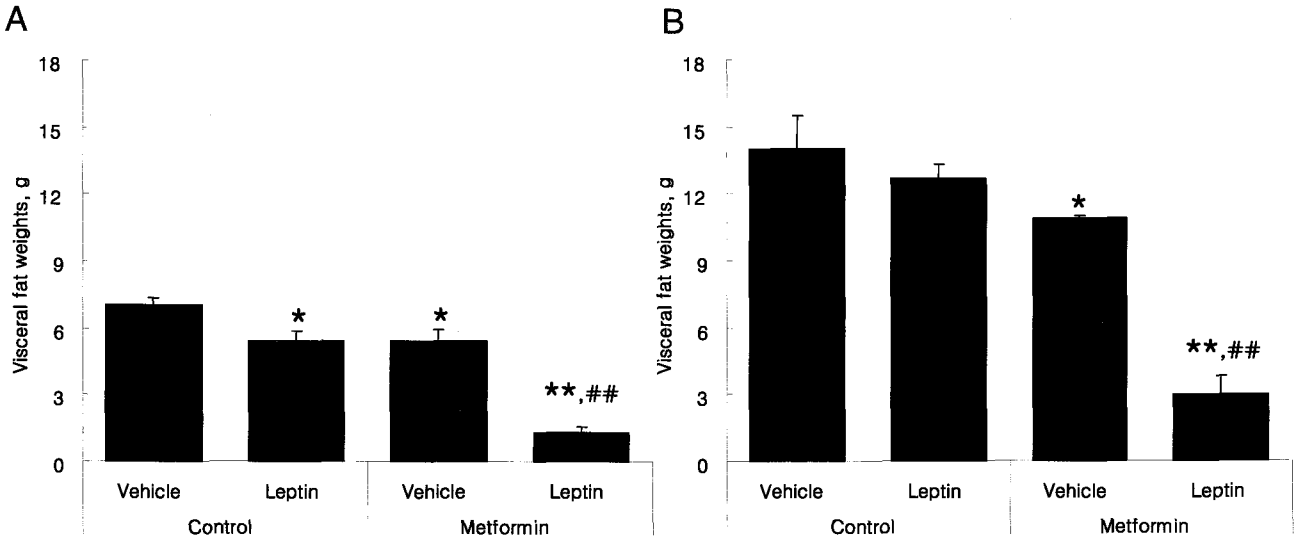

Fig. 5. Visceral fat mass among experimental groups in adult (A) and aged (B) rats. Values are means±S.E. of 6~8 rats per group. *$p<0.05$, **$p<0.01$, vs control vehicle; $^{\#\#}p<0.01$, vs metformin vehicle.

rats, but not in aged rats. Metformin treatment decreased visceral fat mass in aged as well as adult rats. Surprisingly, however, the visceral fat mass was decreased markedly with leptin treatment in both metformin treated adult and aged rats, compared to their control counterparts (Fig. 5).

Serum level of leptin

Serum leptin level was elevated in aged rats compared to adult rats, which poorly responded to leptin treatment. Metformin treatment per se did not significantly decrease serum concentration of leptin in aged rats, however, it was significantly decreased by the addition of leptin (Fig. 6).

DISCUSSION

Aging is associated with various medical problems, such as obesity, hypertension, atherosclerosis, diabetes, and dyslipidemia, which are components of metabolic syndrome. One of the causes of obesity in aging process is leptin resistance, which responds poorly to leptin treatment. Thus, improving leptin sensitivity is essential for the treatment of aging-associated obesity.

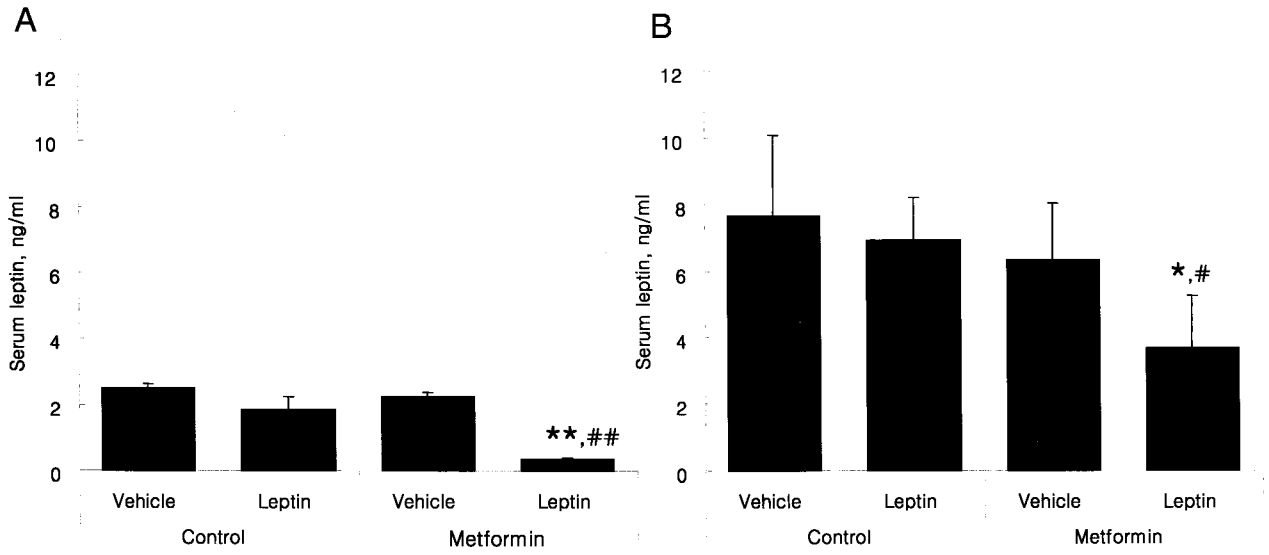

Fig. 6. Serum leptin concentration of experimental groups in adult (A) and aged (B) rats. Values are means±S.E. of 6~8 rats per group. *$p<0.05$, vs control vehicle; #$p<0.05$, vs metformin vehicle.

There have been numerous studies to improve leptin sensitivity, however, only caloric restriction has been found to increase leptin sensitivity in diet-induced obese model (Fernandez-Galaz et al, 2002; Levin & Dunn-Meynell, 2002). We recently reported (Kim et al, 2004) that metformin increases leptin sensitivity in diet-induced obese rats as well as normal rats. Thus, we attempted to evaluate whether metformin could affect leptin sensitivity in aged rat model.

In the present study, we found that anorexic and fat losing effects of i.c.v. leptin were more prominent in metformin treated rats than in untreated adult rats. Moreover, metformin restored leptin sensitivity in aged rats. Interestingly, the effect of metformin on leptin action was more prominent in aged rats than in adult rats. However, we could not offer any explanation on the exact mechanism for augmented anorexic response to metformin in aged rats. Nevertheless, we can only speculate that higher serum leptin level in aged rats is responsible for the augmented anorexic response to metformin, because adequate leptin concentration is required for action of leptin. Actually, in the present study, metformin treatment decreased leptin concentration, suggesting an equilibrizing process resulting from increased leptin sensitivity due to metformin treatment.

The biguanide coumpound metformin has clinically been widely used for treatment of type 2 diabetes for decades (Radziuk et al, 2003). However, the comprehensive molecular mechanism of antihyperglycemic effect of metformin has yet to be established. It has recently been reported that metformin targets AMP-activated protein kinase (AMPK), which is also activated by leptin (Zhou et al, 2001; Fryer et al, 2002; Hawley et al, 2002; Minokoshi et al, 2002; Minokoshi et al, 2004). These results suggest a more delicate interaction taking place between metformin and leptin. It is also possible that metformin affects leptin signalling directly and/or indirectly in the hypothalamus. However, there is only a few studies to indicate direct effect of metformin in the hypothalamus. It is highly possible that transport through blood brain barrier of metformin could not be a limited factor, because metformin reduces androgen level and restores menstrual cycle in polycystic ovarian syndrome (PCOS) patients (Morin-Papunen et al, 1998; Nestler et al, 1998; Glueck et al, 1999; Kolodziejczyk et al, 2000; Moghetti et al, 2000), which is the reason of why metformin is widely prescribed for PCOS patients.

Taken together, the anorexic effect of metformin might be associated with the action of leptin. However, further study is needed to clarify how metformin affects the action of leptin.

In summary, metformin enhances anorexic and fat losing activity of leptin in adult rats and restores leptin sensitivity in aged rats with leptin resistance. The combination therapy with metformin and leptin is recommended for treatment of aging-associated obesity.

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REFERENCES

Cankurtaran M, Halil M, Yavuz BB, Dagli N, Oyan B, Ariogul S. Prevalence and correlates of metabolic syndrome (MS) in older adults. *Arch Gerontol Geriatr* 42: 35–45, 2006

Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 334: 292–295, 1996

Fruehwald-Schultes B, Oltmanns KM, Toschek B, Sopke S, Kern W, Born KJ, Fehm HL, Peters A. Short-term treatment with metformin decreases serum leptin concentration without affecting body weight and body fat content in normal-weight healthy men. *Metabolism* 51: 531–536, 2002

Fryer LG, Parbu-Patel A, Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. *J Biol Chem* 277: 25226–

25232, 2002

Glueck CJ, Fontaine RN, Wang P, Subbiah MTR, Weber K, Illig E, Streicher P, Sieve-Smith L, Tracy T, Kang JE, McCullough P. Metformin reduces weight, centropedal obesity, insulin, leptin, and low-density lipoprotein cholesterol in nondiabetic, morbidly obese subjects with body mass index greater than 30. *Metabolism* 50: 856–861, 2001

Glueck CJ, Wang P, Fontaine R, Tracy T, Sieve-Smith L. Metformin-induced resumption of normal mense in 39 of 43 (91%) previously amenorrheic women with the polycystic ovary syndrome. *Metabolism* 48: 511–519, 1999

Fernandez-Galaz C, Fernandez-Agullo T, Perez C, Peralta S, Arribas C, Andres A, Carrascosa JM, Ros M. Long-term food restriction prevents ageing-associated central leptin resistance in Wistar rats. *Diabetologia* 45: 997–1003, 2002

Halaas JL, Booze C, Blair-West J, Fidahusein N, Denton DA, Friedman JM. Physiological response to long term peripheral and central leptin infusion in lean and obese mice. *Proc Natl Acad Sci* 94: 8878–8883, 1997

Hawley SA, Gadalla AE, Olsen GS, Hardie DG. The antidiabetic drug metformin activates the AMP-activated protein kinase cascade via an adenine nucleotide-independent mechanism. *Diabetes* 51: 2420–2425, 2002

Hundal HG, Ramlal T, Reyes R, Leiter LA. Cellular mechanism of metformin action involves glucose transporter translocation from an intracellular pool to the plasma membrane in L6 muscle cells. *Endocrinology* 131: 1165–1173, 1992

Kay JP, Alemzadeh R, Langley G, D'Angelo L, Smith P, Holshouser S. Beneficial effects of metformin in normoglycemic morbidly obese adolescents. *Metabolism* 50: 1457–1461, 2001

Kim YW, Choi DW, Park YH, Huh JY, Won KC, Choi KH, Park SY, Kim JY, Lee SK. Leptin-like effects of MTII are augmented in MSG-obese rats. *Regul Pept* 127: 63–70, 2005

Kim YW, Kim JY, Park YH, Park SY, Won KC, Choi KH, Huh JY, Moon KH. Metformin restores leptin sensitivity in high fat obese rats with leptin resistance. *Diabetes* 53(Suppl 2): A409, 2004.

Klip A, Guma A, Ramlal T, Bilan PJ, Lam L, Leiter LA. Stimulation of hexose transport by metformin in L6 muscle cells in culture. *Endocrinology* 130: 2535–2544, 1992

Kolodziejczyk B, Duleba AJ, Spaczynski RZ, Pawelczyk L. Metformin therapy decreases hyperandrogenism and hyperinsulinemia in women with polycystic ovary syndrome. *Fertil Steril* 73: 1149–1154, 2000

Levin BE, Dunn-Meynell AA. Reduced central leptin sensitivity in rats with diet-induced obesity. *Am J Physiol Regul Integr Comp Physiol* 283: R941–R948, 2002

Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* 415: 339–343, 2002

Minokoshi Y, Alquier T, Furukawa N, Kim YB, Lee A, Xue B, Mu J, Foufelle F, Ferre P, Birnbaum MJ, Stuck BJ, Kahn BB. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. *Nature* 428: 569–574, 2004

Moghetti P, Castello R, Tosi F, Perrone F, Caputo M, Zanolin E, Muggeo M. Metformin effects on clinical features, endocrine and metabolic profiles and insulin sensitivity in polycystic ovary syndrome: a randomized, double blind, placebo-controlled 6-month trial, followed by open, long-term clinical evaluation. *J Clin Endocrinol Metab* 85: 139–146, 2000

Morin-Papunen LC, Koivunen RM, Tomas C, Ruokonen A, Martikainen HK. Decreased serum leptin concentrations during metformin therapy in obese women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 83: 2566–2568, 1998

Nestler JE, Jakubowicz DJ, Evans WS, Pasquali R. Effects of metformin on spontaneous and clomiphine-induced ovulation in the polycystic ovary syndrome. *N Engl J Med* 338: 1876–1880, 1998

Radziuk J, Bailey CJ, Wiernsperger NF, Yudkin JS. Metformin and its liver targets in the treatment of type 2 diabetes. *Curr Drug Targets Immune Endocr Metab Disord* 3: 151–169, 2003.

Scarpace PJ, Matheny M, Moore RL, Tumer N. Impaired leptin responsiveness in aged rats. *Diabetes* 49: 431–435, 2000

Shek, EW, Scarpace PJ. Resistance to the anorexic and thermogenic effects of centrally administrated leptin in obese aged rats. *Regul Pept* 92(1-3): 65–71, 2000

van den Brandt J, Kovacs P, Kloting I. Metabolic syndrome and aging in Wistar Ottawa Karlsburg W rats. *Int J Obes Relat Metab Disord* 26: 573–576, 2002

Velazquez EM, Mendoza S, Hamer T, Sosa F, Glueck CJ. Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy. *Metabolism* 43: 647–654, 1994

Wiernsperger NF, Bailey CJ. The antihyperglycemic effect of metformin: therapeutic and cellular mechanisms. *Drugs* 58: 31–39, 1999

Zhou G, Myers R, li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated kinase in mechanism of metformin action. *J Clin Invest* 108: 1167–1174, 2001