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## The Chinese *Pueraria* root extract (*Pueraria lobota*) ameliorates impaired glucose and lipid metabolism in obese mice

Jeevan K. Prasain<sup>a</sup>, Ning Peng<sup>b</sup>, Rajani Rajbhandari<sup>a</sup>, and J. Michael Wyss<sup>b,\*</sup>

<sup>a</sup>Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

<sup>b</sup>Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

### Abstract

The incidence of type 2 diabetes and metabolic disease is rapidly increasing, but effective therapies for their prevention and treatment have been poorly tolerated or minimally effective. In this study, chronic administration of kudzu root extract (8 months, 0.2% w/w in diet) decreased baseline fasting plasma glucose (183±14 vs 148±11 mg/dl) and improved glucose and insulin tolerance in C57BL/6J *ob/ob* mice (1.67±0.17 ng/ml [kudzu treated] vs. 2.35±0.63 ng/ml [control]), but such treatment did not alter these parameters in lean control mice. Among the mice on the kudzu supplementation, plasma levels of isoflavone metabolites were significantly higher in *ob/ob* versus lean control mice, and unmetabolized puerarin (11.50±5.63 ng/gram) was found in adipose tissue only in the treated mice. Together, these data demonstrate that a puerarin containing kudzu diet improves glucose and insulin responsiveness in *ob/ob* mice, suggesting that puerarin may be a beneficial adjuvant for treating metabolic disease.

### Keywords

Kudzu; puerarin; glucose metabolism; lipids

### Introduction

The metabolic syndrome affects nearly one-fourth of US adults (~47 million people), and a prominent feature of this syndrome is impaired glucose regulation (Ford et al., 2002, Park et al., 2003). In the metabolic syndrome, insulin resistance, hypertension, obesity and hypercholesterolemia synergize to accelerate the development of cardiovascular disease, stroke and type 2 diabetes (Kraja et al., 2008). Elevated circulating free fatty acids (FFA), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and leptin are associated with obesity and may contribute to insulin resistance (Cohen et al., 1996, Segal et al., 1996, Muller et al., 1997, Summers, 2006). When their storage capacities are exceeded, the excess fat begins to accumulate in adipocytes, which can lead to the formation of specific metabolites that can inhibit insulin signal transduction. Among the fats that accumulate, non-esterified fatty acids, triglyceride, diacylglycerol and ceramide are all associated with insulin resistance.

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\*Corresponding author: J. M. Wyss, 1900 Univ. Blvd./THT 950, Dept. of Cell Biology, Univ. of Alabama at Birmingham, Birmingham, AL35294-0006, jmwyss@uab.edu.

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Sphingolipid ceramide is a putative intermediate linking excess nutrients (i.e. saturated fatty acids) and inflammatory cytokines (e.g., TNF $\alpha$ ) to insulin resistance. Moreover, ceramide has been shown to be toxic in a variety of different cell types (e.g. pancreatic beta-cells, cardiomyocytes, etc. (Summers, 2006, Teruel et al., 2001).

Recently, there has been a growing interest in the ability of dietary polyphenols to reduce these interacting factors (Cefalu et al., 2008). In rats, we and others have shown that several dietary polyphenolic compounds can decrease blood pressure and serum total cholesterol and improve insulin signaling (Boue et al., 2003, Hsu et al., 2003, Meezan et al., 2005, Peng et al., 2005, Carlson et al., 2008). Kudzu root (Radix pueraria from *Pueraria lobota*), which has recently become commercially available in Western dietary supplements, is widely used in traditional Chinese medicine, and it is a rich source of isoflavone glucosides. Common isoflavones of kudzu root include puerarin (daidzein 8-*C*-glucoside), daidzin (daidzein 7-*O*-glucoside), daidzein, genistein and formononetin. These isoflavones have been associated with antioxidant, anti-dipsotropic and other pharmacological effects (Lee, 2004, Keung and Vallee, 1998, Zhang et al., 2010). Among the isoflavones in kudzu root, puerarin is the most abundant (~23% w/w) and has attracted considerable attention, because of its putative ability to protect against metabolic disorders (Meezan et al., 2005, Xu et al., 2005). However, the mechanisms underlying the beneficial actions of kudzu isoflavones are not clear.

The present study tested the effects of dietary kudzu supplementation on glucose and lipid metabolism in genetically obese animals. ESI-MS and MS/MS strategies were established to profile lipid molecular species in adipose tissue, so as to determine lipid changes that occur in obese and lean animals after chronic administration of kudzu. The results obtained from these studies provide insights into potential mechanisms by which kudzu extract, (likely via puerarin) can alter the course of metabolic disease.

## Materials and methods

### Reagents

Methanol (HPLC grade) and chloroform were from Fisher Scientific (Fair Lawn, NJ). Lipid standards were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Puerarin root extract was obtained in the powder form from AMAX NutraSource, Eugene, OR.

### Diet Preparation

The experimental diet was made by adding powdered Pueraria root extract (0.2% w/w) to AIN-93M, a phytoestrogen-free diet (see Reeves et al, 1993 for a full description of this diet). The combination was blended overnight in a rotating mixer and pelleted (Test Diet, Richmond, IN). The control diet was a similarly pelleted AIN-93M diet with no additions (Test Diet, Richmond, IN). The diets were tested monthly to insure consistency of isoflavone content. The kudzu root extract concentration in the diet was chosen based on our previous studies (Peng et al., 2009).

### Animal experiments

Male obese mice (C57BL/6J-*ob/ob*) and their lean controls (C57BL/6J-*+/+*) were purchased from the Jackson Laboratory (Bar Harbor, ME) at 4 weeks of age. They were housed three per cage at constant humidity (65  $\pm$  5%), temperature (24  $\pm$  1  $^{\circ}$ C), and light-dark cycle (0600–1800 h, lights on), and allowed *ad libitum* access to tap water and diet throughout the experimental protocols. All experimental procedures were conducted under the oversight and approval of the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham and in accordance with National Institutes of Health guidelines and the *Guide to the Care and Use of Laboratory Animals*.

### **Kudzu administration, general lipid/fat assessments, blood pressure and heart rate glycemc control and insulin sensitivity**

To investigate whether long-term exposure to kudzu root extract alters plasma glucose and insulin tolerance, mice were maintained on a polyphenol-free AIN 93M diet with or without the addition of 0.2% kudzu root extract for 8 months (9 mice per diet group per strain). Blood pressure and heart rate were measured indirectly, using a Hatteras tail cuff system (MC 4000) once every two months.

After 7 months on the diets, *in vivo* body composition (total body fat and lean tissue) of mice was determined using an EchoMRI™ 3-in-1 quantitative magnetic resonance (QMR) machine (Echo Medical Systems, Houston, TX). A system test was performed using a known fat standard prior to the measurements being taken. Mice were weighed and then placed into a clear holding tube capped with a stopper that restricted vertical movement, but allowed constant airflow. The tube was inserted into the machine and the mouse scanned using the Normal Precision mode.

After the 8 month on the diets, glucose tolerance was tested. Mice were fasted overnight and blood glucose measurements were performed the following morning. Mice were lightly anesthetized (isoflurane), and a tail blood micro sample was taken for a baseline blood glucose concentration measurement using a blood glucose monitoring system (Prestige Smart System, CVS). Mice then received an oral glucose gavage (2 grams glucose/kg body weight). On an alternate day, to determine whether chronic dietary kudzu extract alters insulin sensitivity, mice received a bolus injection of insulin (0.75 U/kg, ip., bovine insulin, Sigma, St Louis, MO). Blood glucose was measured at 15, 30, 45, 60, 90 and 120 minutes after the glucose/insulin challenge.

At the end of the study, the mice were euthanized by cervical dislocation under isoflurane anesthesia, and blood was collected via heart puncture using heparin as anticoagulant. Adipose tissue was isolated and immediately frozen in liquid nitrogen and stored at  $-20^{\circ}\text{C}$  until used.

Plasma was isolated by centrifugation of the blood at  $3,000 \times g$  for 5 min at stored at  $-20^{\circ}\text{C}$ . Plasma lipids were analyzed using Vitros DT60 [Ektachem] analyzer, (Ortho-Clinical Diagnostics, Rochester, NY). Adipose tissue samples (wet weight 33–35 mg) were added to (1 ml) methanol containing 1% acetic acid and homogenized in a mortar. The samples were vortex mixed and centrifuged at  $3,000 \times g$  for 10 min and the supernatant was removed and dried under air and reconstituted in 80% methanol in water (200  $\mu\text{l}$ ).

For individual lipid assessments, lipids were extracted following Bleigh Dyer method (21). Briefly, adipose tissue (30–35 mg) was homogenized in a mortar filled with liquid nitrogen and extracted with 1 ml water, 2.5 ml of methanol and 1.25 ml of chloroform. The mixture was sonicated with 4 bursts, and added 1ml of water, and 1.25 ml of chloroform. After vigorous shaking and centrifugation at  $3,000 \times g$  for 5 min, the supernatant was removed and the bottom aqueous layer was collected. Supernatant was extracted again with 1.25 ml of chloroform. The extracted solution was evaporated to dryness and reconstitute with 100  $\mu\text{l}$  of chloroform:methanol (1:1 v/v).

Lipids were analyzed by ESI-MS/MS using an API 4000 (Applied Biosystems/MDS Sciex, Concord, Ontario, Canada) triple quadrupole mass spectrometer. Samples (5  $\mu\text{l}$ ) were directly infused into the electrospray source using a Shimadzu Prominence HPLC with auto sampler (Shimadzu Scientific Instruments, Inc. Columbia, MD). The samples were infused into the mass spectrometer using a solvent mixture of chloroform-methanol (1:2, v/v) containing 0.1% formic acid. The analysis was performed in positive ion mode electrospray

ion (ESI-MS) source and precursor ion scans  $m/z$  264 and 282 and multiple reaction ion monitoring (MRM) mass transition  $m/z$  564/282 (ceramides), and precursor ion scan  $m/z$  184 lysophosphocholine (LPC), were used.

MSMS parameters were optimized to obtain best sensitivity. The declustering potential and temperature were set at 35 V and 250 °C, respectively. The collision energy was optimized and set at 35 V. The MS/MS system was controlled by BioAnalyst 1.4.2 software.

### Plasma isoflavone analysis

Isoflavone analysis was performed following our published method (Prasain et al., 2010). Briefly, each serum sample (200  $\mu$ l) was subjected to enzymatic hydrolysis of the isoflavone glucuronides and sulfates for the total isoflavone determination and extracted into diethyl ether. After concentrating samples to dryness, they were dissolved in methanol-water (80:20, v/v) prior to LC-MS/MS analysis. Sample preparation and quantification of puerarin in biological samples were performed as described before (Prasain et al., 2007).

### Statistical analysis

All experimental data were evaluated by two-way analysis of variance (ANOVA) followed by post hoc Tukey's test to determine the source of main effects and interactions (SPSS, Chicago, IL). The significance criterion for all experiments was  $p < 0.05$ . All data are reported in the results section as mean  $\pm$  standard error.

## Results

### General health of mice in each group

Kudzu did not significantly affect the body weights of the mice in the study; however, as expected the ob/ob mice weighed nearly twice as much as the lean control mice (Table 2). Dietary kudzu supplementation did not affect food consumption in either lean control or ob/ob mice (data not shown). Further, QMR analysis at the end of the study demonstrated no significant differences between groups in fat or lean mass (Table 2). Plasma total cholesterol concentrations were not significantly different between groups; total cholesterol was highest in the ob/ob mice on kudzu (Table 2). Plasma triglycerides were also not different between groups (Table 2). Blood pressure and heart rate were lower in the ob/ob than lean control mice, but kudzu affected neither index (Table 2).

### Glucose metabolism

In the present study in ob/ob mice, chronic dietary supplementation with kudu root extract (containing ~23% puerarin, w/w) significantly decreased baseline fasting glucose (183 $\pm$ 14 vs. 148 $\pm$ 11 mg/dl) and improved glucose tolerance and insulin sensitivity. On control diet, the ob/ob (compared to the lean control) mice displayed significant impairment in glucose tolerance, especially during the second half of the tolerance curve (after 45 m; Fig. 1). Ob/ob mice on the kudzu dietary supplementation showed significantly improved glucose tolerance, reaching the response levels of control mice by 45 minutes after glucose administration. In lean control mice, the kudzu supplementation modestly improved glucose tolerance at most time points.

Kudzu supplementation reduced plasma insulin concentration in ob/ob mice (1.67 $\pm$ 0.17 ng/ml [kudzu treated] vs. 2.35 $\pm$ 0.63 ng/ml [control]). To determine whether dietary kudzu supplementation affected insulin-induced glucose clearance, insulin tolerance tests (ITT) were performed on kudzu-treated ob/ob and lean control mice. Insulin challenge (0.75 u/kg) significantly decreased blood glucose levels in all groups. However, in ob/ob mice, but not in lean mice, dietary kudzu root extract caused a significantly greater decrease in plasma

glucose concentration (Fig. 2). Further, for the initial 45 minutes after the insulin challenge, kudzu treated ob/ob mice responded nearly identically to lean control mice (Fig. 2); however, by 60 m after insulin challenge, the ob/ob mice that were treated with dietary kudzu returned to the levels of the non-kudzu diet ob/ob mice, indicating that insulin sensitivity was largely but not completely restored by the kudzu supplementation.

### Lipid metabolism

In ob/ob mice, kudzu treatment had no significant effect on plasma adiponectin levels ( $13.79 \pm 0.52$   $\mu\text{g/ml}$ , vs.  $12.84 \pm 0.66$   $\mu\text{g/ml}$  control), and the concentrations were similar to those in lean mice on the control diet (Fig. 3). Electrospray tandem mass spectrometry (ESI-MS/MS) analysis of ceramides and lysophosphocholine (LPC) in adipose tissues from kudzu treated and control animals was performed. Since ceramides in positive ion mode MS/MS generate characteristic product ions of  $m/z$  264 and 282, precursor ion scans of  $m/z$  264 and  $m/z$  282 were used for detecting ceramides in the adipose tissue. Fig. 4A shows the precursor ion chromatogram with  $m/z$  282 and the ion  $m/z$  564 (C18:1) is the major ceramide in the extract. To increase the sensitivity and specificity, MRM analysis with different mass transitions was performed (data not shown). Although not conclusive, chronic dietary kudzu supplementation reduced elevated adipose C18:1 ceramide in obese animals (Fig. 4B). Precursor ion scan of  $m/z$  184 in positive ion mode demonstrated no significant difference in lysophosphocholine (LPC) in adipose tissue of ob/ob and lean mice, irrespective of their diet (Fig. 5).

### Bioavailability of isoflavones in obese and lean animals

We previously reported a HPLC fingerprint analysis of Pueraria root extract used in this study (Peng et al., 2009). Based on this study, puerarin was the most abundant isoflavone (25.3%) followed by daidzin (7.07%) and daidzein (0.8%) in the root extract. The bioavailability of kudzu isoflavones and their metabolites in plasma and adipose tissue (LC-MS/MS analysis) was significantly higher in obese than lean control mice (Table 1). In contrast to the results of acute administration of puerarin, in which puerarin is the dominant isoflavone, in mice treated chronically with kudzu root extract, equol was the most abundant plasma metabolite  $29,300 \pm 3,457$  nM, followed by daidzein ( $883 \pm 185$  nM) and dihydrodaidzein ( $751 \pm 269$  nM; Table 1). The plasma concentration of unmetabolized puerarin in the treated group was very low ( $10.44 \pm 2.5$  nM). No isoflavone metabolites were detected in the control groups. Intact puerarin (Fig. 6) was present in adipose tissue ( $11.50 \pm 5.63$  ng/gram), potentially protecting the tissue against reactive lipids.

### Discussion

This current data demonstrate that chronic kudzu isoflavones can improve glucose and lipid homeostasis and insulin sensitivity in ob/ob mice, an animal model of type 2 diabetes mellitus. This extends our previous studies, which demonstrate that acute administration of puerarin significantly improves glucose tolerance in ob/ob mice (Meezan et al., 2005). It is notable that the kudzu supplementation significantly altered glucose metabolism, but it did not appear to affect cardiovascular control, or bodyweight or body fat/lean mass. This is in contrast to the situation in the rat, in which body weight and cardiovascular function is improved by the kudzu supplementation (Peng, et al., 2009). The lack of these latter effects of kudzu supplementation in the ob/ob is not entirely unexpected, since the driving force in physiology of the ob/ob mouse is the lack a functional leptin system, a system that the kudzu root extract or puerarin appear to have little influence over. Total plasma cholesterol and HDL were increased and LDL was lowered by the kudzu supplementation in the ob/ob but not lean control mice. Despite kudzu root extract's apparent lack of beneficial action on the leptin pathway, the supplement clearly has beneficial effects on glucose/insulin regulation

and lipids in the ob/ob, and thus, these findings, paired with our previous reports in the rat, suggest the effectiveness of kudzu polyphenols in different models of glucose/insulin dysregulation.

Kudzu root extract contains both puerarin and daidzin. We have shown that acute administration of daidzin has an adverse effect on glucose tolerance, whereas acute administration of either kudzu root extract or puerarin improves glucose tolerance (Meezan et al., 2005). The present results demonstrate that chronic feeding with the whole kudzu root extract improves glucose tolerance to about the same extent as previously observed following acute treatment with either puerarin or kudzu root extract. Thus, any adverse effects of dietary daidzin on glucose handling appear minimal in mice fed kudzu root extract. The role that circulating equol and daidzien have in the chronic effects of kudzu root extract supplementation requires further study.

It is noteworthy that in the lean mice the kudzu root extract significantly lowered baseline plasma glucose concentration, but it did not significantly affect the glucose tolerance curve (Fig. 1). Insulin challenge resulted in a larger decrease in blood glucose in kudzu-treated compared to the control diet ob/ob mice, but the treatment had no effect on lean control mice (Fig. 2). Thus, the chronic supplementation improves both glucose tolerance and insulin sensitivity in ob/ob mice, as it also does in the rat model (24). The failure of the supplementation to improve insulin tolerance in lean mice is likely the result of a “floor” effect. Plasma insulin levels in ob/ob mice were lowered by kudzu treatment, suggesting that kudzu supplementation both improves glucose tolerance and reduces peripheral insulin resistance, without increasing circulating insulin.

The finding that kudzu root extract can favorably decrease plasma ceramide concentration in ob/ob, despite its lack of effect on total cholesterol is potentially an important mechanism in its ability to improve glucose/insulin regulation. Previous *in vitro* studies have demonstrated that some lysophosphatidylcholine (LPC) species activate adipocyte glucose uptake and lower blood glucose levels in murine models of diabetes (Yea et al., 2009), and LPC appears to have pathophysiological functions including inflammation (Oestvang et al., 2011). We therefore, analyzed adipose samples for LPC by tandem mass spectrometry in positive ion mode. Precursor ion scan  $m/z$  184 is very specific for identifying choline-containing phospholipids in positive ion mode. In this study, ion intensities of LPC molecular species in adipose tissue of lean and obese were not significantly different, irrespective of diet (Fig. 5).

Insulin resistance is associated with dysregulation of lipid metabolites including triglyceride, ceramides and fatty acids. Excessive accumulation of ceramide contributes to metabolic dysfunction, whereas adiponectin improves metabolic function (Holland and Scherer, 2009). The present study tested the hypothesis that dietary kudzu supplementation increases plasma adiponectin (the major adipokine circulating in plasma in different isoforms) and decreases ceramide in adipocytes. The current data demonstrate no effect on of the dietary extract on circulating adiponectin; although not conclusive quantitatively, kudzu supplementation decreases C18:1 ceramide levels in adipose tissue. Ceramide can directly inhibit the insulin receptor subunit 1 (IRS1; 28), and thus, the ability of dietary kudzu root extract to improve glucose and insulin responses may directly relate to its actions on ceramide.

Although drug absorption is not directly affected by obesity, obesity alters the distribution, protein binding, metabolism and renal excretion of drugs (Blouin et al., 1987). In the present study, circulating isoflavones were much higher in ob/ob compared to lean control mice. There has been almost no documentation specifically addressing the impact of obesity on isoflavone metabolism. Following acute administration of puerarin or kudzu root extract, the puerarin is rapidly absorbed into the blood with plasma levels peaking about 45 min after

administration (Prasain et al., 2007, Prasain et al., 2004). Thereafter, the circulating puerarin is rapidly excreted in the urine and only low concentrations of daidzein and equal remain present in the blood. In our previous studies in rats, oral administration of puerarin produced a high concentration of intact puerarin in the urine collected within the first 4 h (Prasain et al., 2004). This indicates that puerarin is rapidly absorbed in the small intestines. After 4 h, the concentration of intact puerarin decreased substantially while the concentrations of daidzein, dihydrodaidzein, and equol were increased in the urine samples. By 72 h equol was the most abundant metabolite in the urine (Prasain et al., 2004). Since equol is produced after reductive metabolism of daidzein by intestinal bacteria, these results suggest that puerarin is slowly hydrolyzed to daidzein by bacterial enzymes in the large intestine and subsequently reduced to dihydrodaidzein and equol. Chronic feeding studies with puerarin versus kudzu root extract will assist in understanding which of these mechanisms is primarily responsible for the high circulating equol concentrations in mice. Further, our previous studies indicate that puerarin is in relatively high concentration in some organs after both acute and chronic feeding (e.g., lung, brain, heart, and kidney) (Prasain et al., 2009). It will be important in the future to determine if the effects of kudzu root extract and puerarin are, at least in part, due to the concentration/sequestration of puerarin into these organs. The current results open a new discussion into the mechanisms by which isoflavone metabolisms have long- and short-term effects on insulin resistance, obesity and other physiological characteristics.

## Conclusion

Despite our incomplete understanding of mechanisms involved in beneficial effects of kudzu isoflavones, the results of this study clearly demonstrate that chronic feeding of puerarin containing kudzu root extract improves glucose and insulin responsiveness in ob/ob mice. Further, this study clearly demonstrates that compared to lean controls, insulin resistant obese animals display significantly higher circulating isoflavones concentrations and administration of a complex botanical (i.e., kudzu root extract) leads to different mixtures of circulating isoflavones, depending on the post-administration time that is tested. Thus, the assessment of the active mechanisms of complex botanicals must consider differential actions due to these changes in circulating isoflavones.

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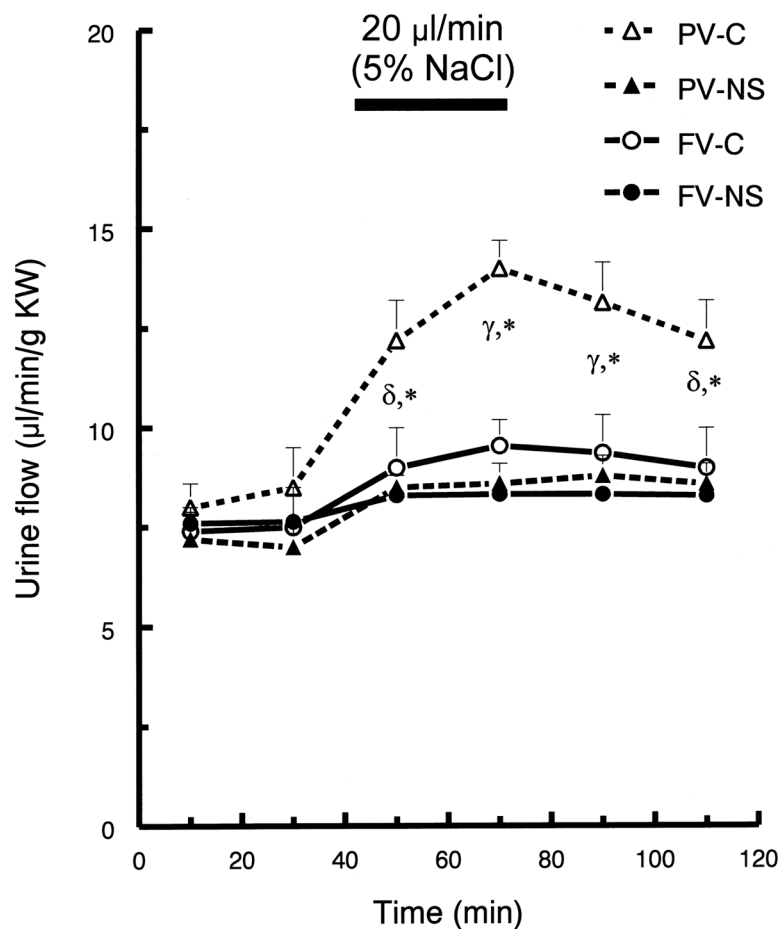
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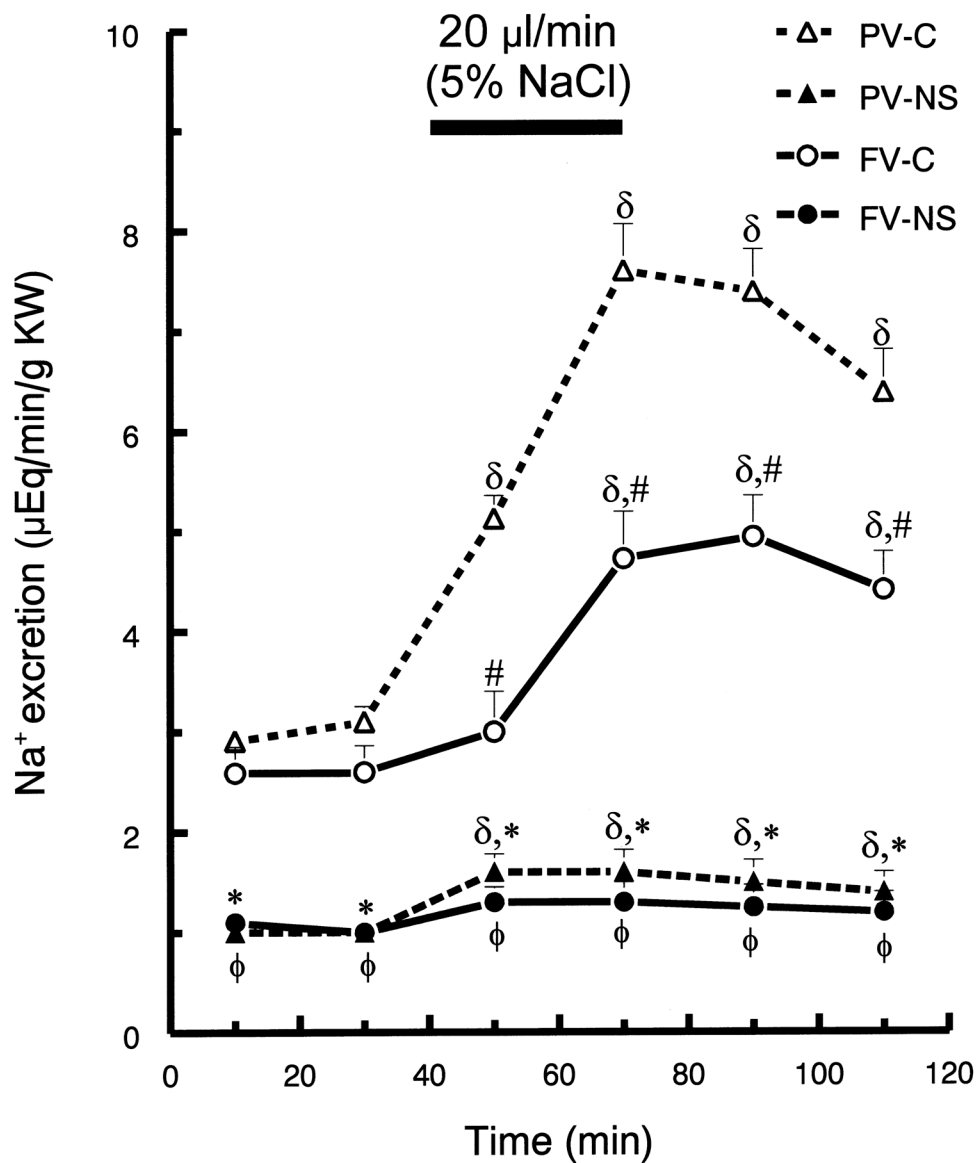
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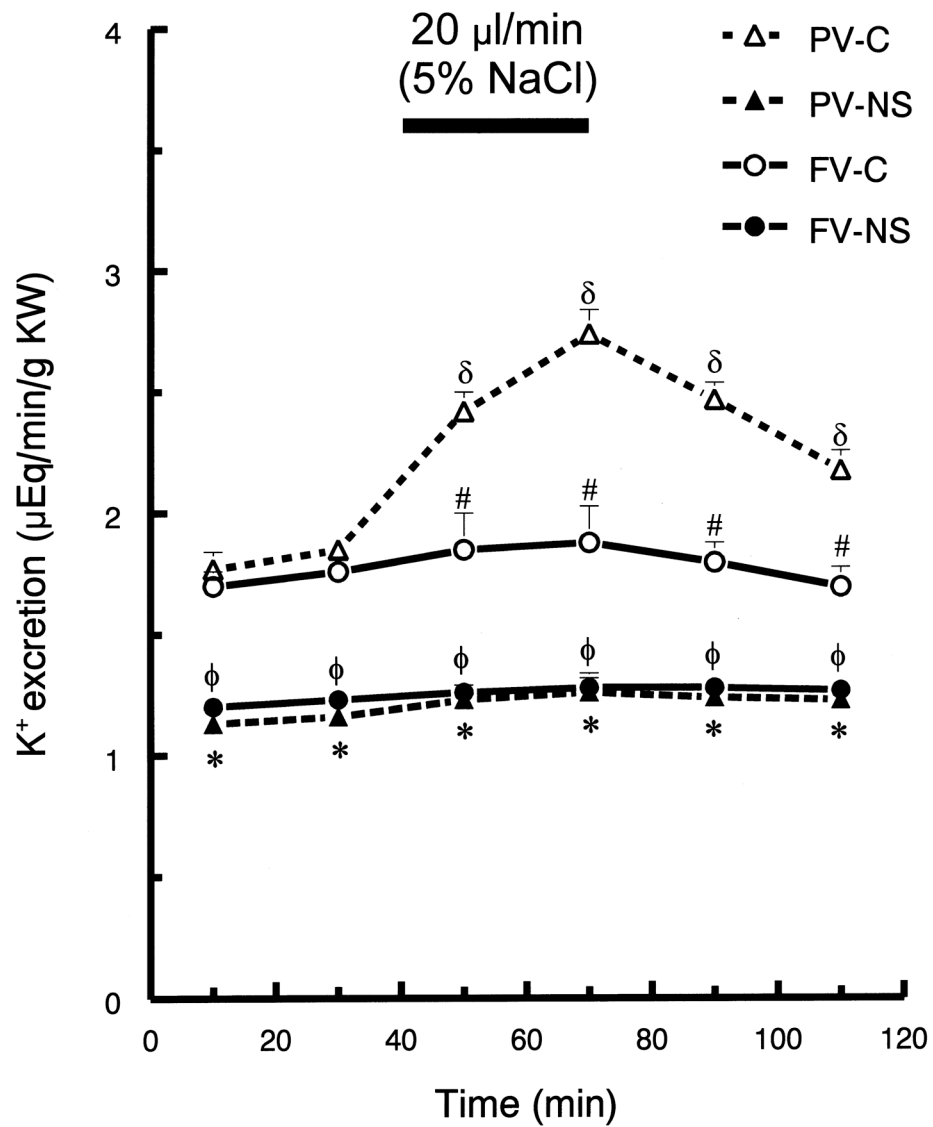
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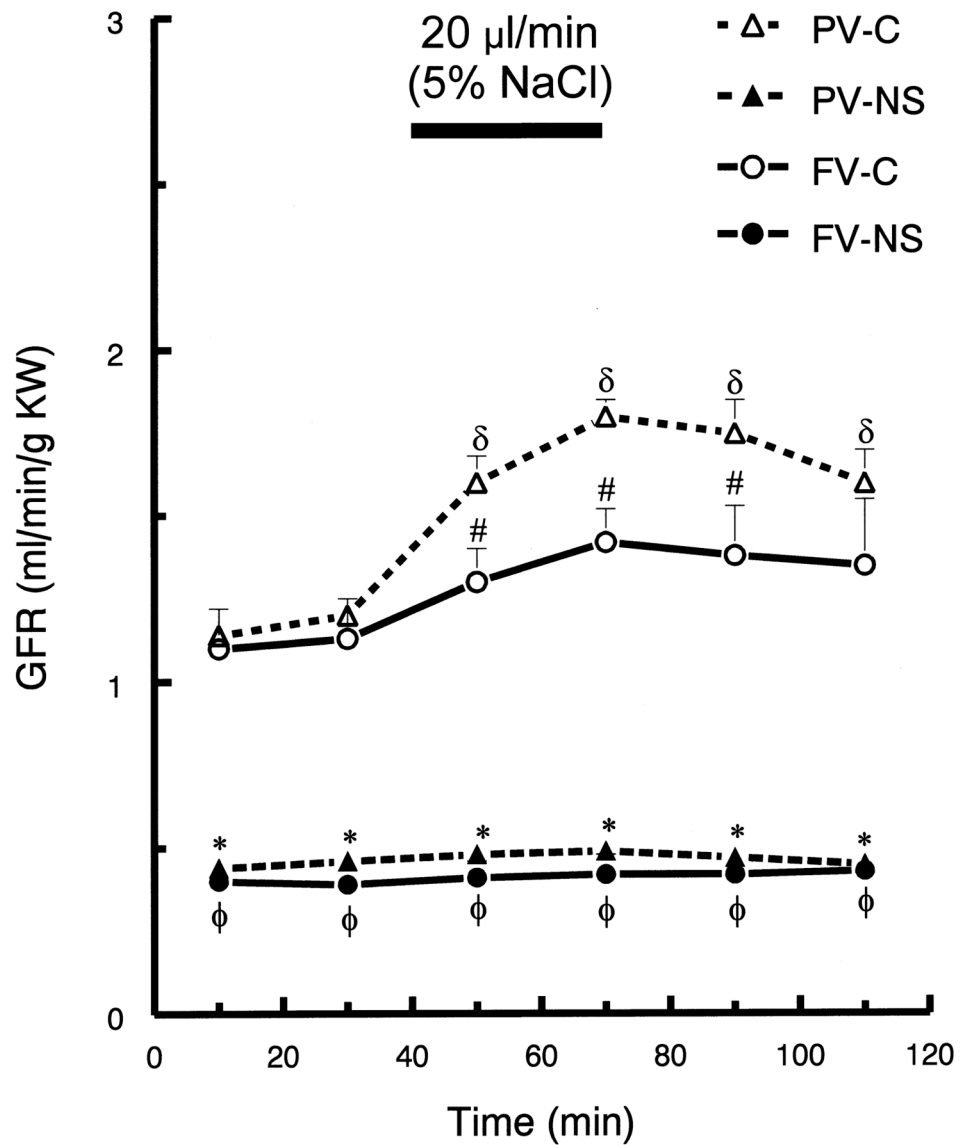
**Fig. 1.** Chronic administration of dietary kudzu root extract improves oral glucose tolerance in C57BL/6J-ob/ob mice. Values are given as means  $\pm$  SEM. #  $p < 0.05$  for both ob/ob groups compared to both lean control groups; \*  $p < 0.05$  for ob/ob on control diet compared to all other groups;  $p < 0.05$  for kudzu fed, compared to control diet fed, lean control mice for all time points except 30 min. Initial plasma glucose levels were significantly different for all four groups. OB-NK, ob/ob mice on control diet, OB-K, ob/ob mice on kudzu diet, Lean-NK, lean mice on control diet.



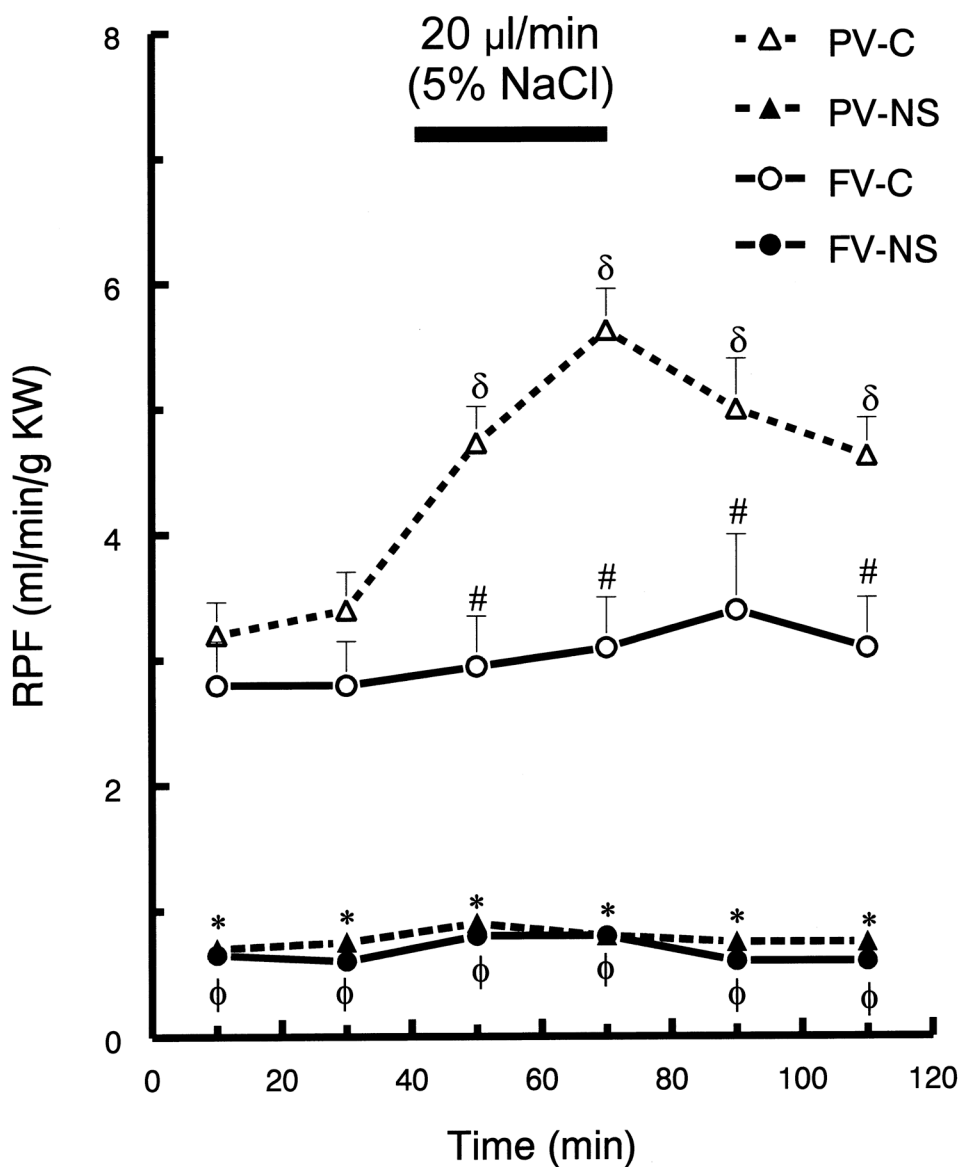
**Fig. 2.** Chronic administration of kudzu root extract improves insulin tolerance in C57BL/6J-ob/ob mice, but not lean control mice. Mice received a bolus injection of insulin (0.75 U/kg, ip) and blood glucose was measured at 15, 30, 45, 60, 90 and 120 minutes after insulin challenge. Values are given as means  $\pm$  SEM. \*  $p < 0.05$  for ob/ob on control diet compared to all other groups. #  $p < 0.05$  for ob/ob groups compared to lean control groups. Initial plasma glucose levels were significantly higher in ob/ob on the control diet versus all other groups for all four groups. OB-NK, ob/ob mice on control diet, OB-K, ob/ob mice on kudzu diet, Lean-NK, lean mice on control diet.



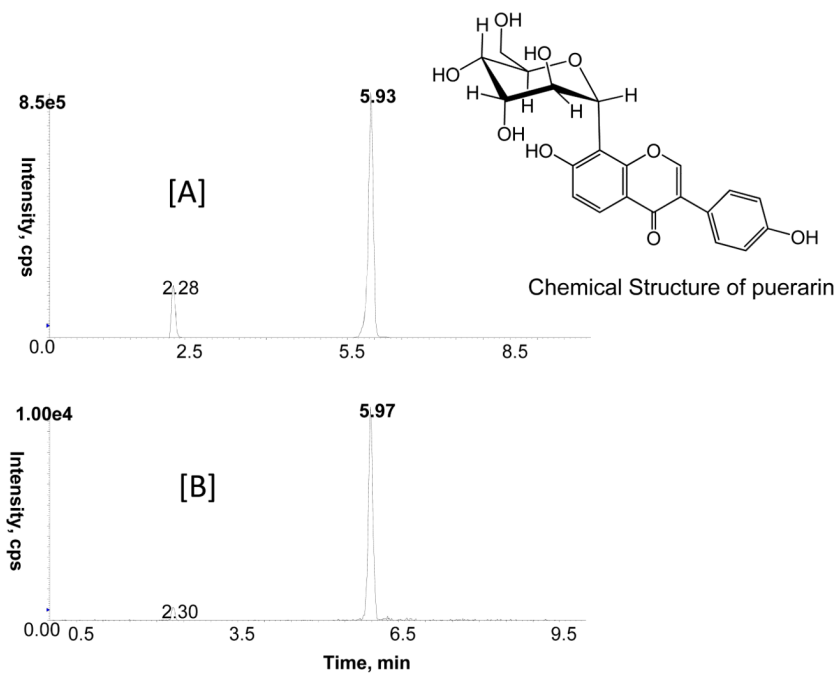
**Fig. 3.** Plasma adiponectin concentration was not significantly different in the three groups tested (ob/ob control diet (OB-NK), ob/ob on kudzu root extract diet (OB-K) and lean mice on control diet (Lean-NK) mice).



**Fig. 4.** Representative precursor ion scan  $m/z$  282 [A] of adipocyte lipid extract. MRM chromatograms [B] showing adipocyte ceramide C18:1 in ob/ob mice on control diet [OB-NK], ob/ob mice on kudzu diet [OB-K] and lean control mice on control diet [Lean-NK]. Mass transition  $m/z$  564/282 was used. Note the differences in the Y-axes (Intensity) scales.



**Fig. 5.** Lysophosphocholines in adipose tissue of ob/ob mice on kudzu diet [OB-K], ob/ob mice on control diet [OB-NK] and lean control mice on control diet [lean-NK]. Precursor ion scan  $m/z$  184 was used to detected molecular species of LPC. Major LPC  $m/z$  496 (16:0),  $m/z$  520 (18:20),  $m/z$  524 (18:0), and  $m/z$  538 (20:0) were identified.



**Fig. 6.** MRM ion chromatogram for puerarin (retention time 5.93 min) with mass transition  $m/z$  416/267 at 1  $\mu\text{g/ml}$  [A]; methanolic extract of adipose tissue chronically treated with kudzu extract in the diet [B].

**Table 1**

Mean  $\pm$  SEM concentration for kudzu isoflavone levels in rat plasma samples (nM)

	<b>Equol</b>	<b>daidzein</b>	<b>puerarin</b>	<b>DHD</b>	<b>formononetin</b>
OB-K	29300 $\pm$ 3457	883 $\pm$ 185	10.44 $\pm$ 2.56	751 $\pm$ 269	143 $\pm$ 68
Lean-K	8286 $\pm$ 430	208 $\pm$ 61	4.57 $\pm$ 1.40	65 $\pm$ 36	ND

DHD = dihydrodaidzein

ND = not detected



**Table 2**

General physiological characteristics of ob/ob (OB) and lean control (C) mice on either a phytoestrogen-free diet (NK) or a diet supplemented with 0.2% (w/w) kudzu root extract.

	OB-NK	OB-K	C-NK	C-K					
BW (g)	70.9	72.7	37.3	1.1	37.1	1.3	$p < 0.05$ OB vs. C		
Fat mass (%; QMR)	34.6	2.3	36.2	2.7	31.3	1.5	32.7	1.9	ns
Lean mass (%; QMR)	22.5	1.1	23.2	1.7	24.1	1.8	25.8	1.8	ns
Total Cholesterol (mg/dl)	212	19	245	30	180	31	172	29	ns
Triglycerides (mg/dl)	83	6	91	5	86	6	87	5	ns
BP (mm Hg; systolic)	116	2	114	4	126	2	129	3	$p < 0.05$ OB vs. C
HR (beats per minute)	619	36	572	37	692	21	680	21	$p < 0.05$ OB vs. C

The data are mean  $\pm$  SE. Significant differences between groups (post hoc after significant 1-way ANOVA) are noted to the right of the table.

BW = Body Weight; Fat and lean mass by QMR; Cholesterol, HDL, LDL and triglycerides; systolic Blood Pressure (BP) and heart rate (HR) measured by tail cuff plethysmography.