Female PAPP-A knockout mice are resistant to metabolic dysfunction induced by high-fat/high-sucrose feeding at middle age

Cristal M. Hill · Oge Arum · Ravneet K. Boparai ·
Feiya Wang · Yimin Fang · Liou Y. Sun ·
Michal M. Masternak · Andrzej Bartke

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Abstract Longevity and aging are influenced by common intracellular signals of the insulin/insulin-like growth factor (IGF)-1 pathway. Abnormally high levels of bioactive IGF-1 increase the development of various cancers and may contribute to metabolic diseases such as insulin resistance. Enhanced availability of IGF-1 is promoted by cleavage of IGF binding proteins (IGFBPs) by proteases, including the pregnancy-associated plasma protein-A (PAPPA). In vitro, PAPP-A is regulated by pro-inflammatory cytokines (PICs) such as interleukin (IL)-6 and tumor necrosis factor (TNF). Mice born with deficiency of the Papp-a gene (PAPP-A knockout (KO) mice) live ~30–40 % longer

than their normal littermates and have decreased bioactive IGF-1 on standard diets. Our objective was to elucidate how the effects of high-fat, high-sucrose diet (HFHS) promote obesity, induce metabolic dysfunction, and alter systemic cytokine expression in PAPP-A KO and normal mice. PAPP-A KO mice fed HFHS diet for 10 weeks were more glucose tolerant and had enhanced insulin sensitivity compared to normal mice fed HFHS diet. PAPP-A KO mice fed HFHS diet had lower levels of pro-inflammatory cytokines (IL-2, IL-6, and TNF- α) compared to normal mice fed the same diet. However, anti-inflammatory cytokine levels (IL-4 and adiponectin) were higher in PAPP-A KO mice fed

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C. M. Hill · A. Bartke
Department of Medical Microbiology, Immunology, and Cell
Biology, Southern Illinois University School of Medicine,
Springfield, IL, USA

A. Bartke
e-mail: abartke@siumed.edu

M. M. Masternak
Burnett School of Biomedical Sciences, College of Medicine,
University of Central Florida, Orlando, FL, USA
e-mail: michal.masternak@ucf.edu

C. M. Hill (✉) · O. Arum · R. K. Boparai · F. Wang ·
Y. Fang · L. Y. Sun · A. Bartke
Geriatrics Research Laboratory, Department of Internal
Medicine, Southern Illinois University School of Medicine,
P.O. Box 19628, Springfield, IL 62794, USA
e-mail: chill@siumed.edu

O. Arum
e-mail: oge.arum@gmail.com

R. K. Boparai
e-mail: nonaboparai@gmail.com

F. Wang
e-mail: fwang@siumed.edu

Y. Fang
e-mail: yfang@siumed.edu

L. Y. Sun
e-mail: lsun@siumed.edu

O. Arum
631 N. 6th St., Springfield, IL 62702, USA

HFHS diet compared to normal mice fed HFHS. Circulating PAPP-A levels were elevated in normal mice fed an HFHS diet compared to normal mice fed a standard, low-fat, low-sucrose (LFLS) diet. Indirect calorimetry showed, at 10 weeks of feeding HFHS diet, significantly increased oxygen consumption (VO_2) in PAPP-A KO mice fed HFHS diet compared to normal mice fed the same diet. Furthermore, respiratory quotient (RQ) was significantly lower in PAPP-A KO mice fed HFHS diet compared to normal (N) mice fed HFHS diet indicating PAPP-A KO mice fed HFHS diet are able to rely on fat as their primary source of energy more so than normal controls. We conclude that PAPP-A KO mice are resistant to the HFHS diet induction of metabolic dysfunction associated with higher levels of anti-inflammatory cytokines and a remarkably metabolic flexible phenotype and that some of the effects of HFHS diet in normal animals may be due to increased levels of PAPP-A.

Keywords High-fat/high-sucrose diet · Immune response · Insulin/IGF-1 signaling · Longevity · Metabolic flexibility · PAPP-A KO mice

Introduction

Longevity and aging are strongly associated with insulin-like growth factor-1 (IGF-1) and insulin signaling, which share common intracellular pathways (Milman et al. 2014; Berryman et al. 2008; Brown-Borg et al. 1996; Bartke 2003; 2005). Effects of IGF-1 resemble the effects of insulin, and IGF-1 generally promotes insulin signaling in the cell (Blundell and Humbell 1980). Abnormally high levels of bioactive IGF-1 increase the development of various cancers and may contribute to metabolic diseases such as insulin resistance (Braun et al. 2011; Combs 2008). Availability of bioactive IGF-1 depends on the levels of IGF-1-binding proteins (IGFBPs) and can be enhanced by cleaving these proteins with proteases, including the pregnancy-associated plasma protein-A (PAPP-A). Pregnancy-associated plasma protein-A is a metalloprotease that is not specific to pregnancy but synthesized in many cells including osteoblasts, fibroblasts, and smooth muscle cells (Conover et al. 2001, 2008). This protease is also found at low levels in ovarian follicles, follicular fluid, luteal cells, and fallopian tubes of non-pregnant women and in the seminal vesicles and seminal fluid of males (Conover et al. 2001). The activity of the

PAPP-A protease increases the levels of bioactive IGF-1 by cleaving IGFBPs of the IGF system, specifically IGFBP-4 and IGFBP-5, and thus promotes insulin/IGF-1 cell signaling.

Conover and colleagues reported that mice born with a deficiency of *Papp-a* live ~30 % longer than their normal littermates, have reduced bioactive IGF-1, and exhibit “mild-dwarfism” at birth—being 60 % smaller when compared to normal littermates and 40 % of body weight compared to normal littermate during development (Conover et al. 2001; Conover et al. 2004; Conover and Bale 2007). Other types of mice with increased lifespan include Ames dwarf (Brown-Borg et al. 1996), Snell dwarf (Flurkey et al. 2002), and growth hormone receptor/growth hormone binding protein knockout (GHR-KO) (Coschigano et al. 2003) mice. While PAPP-A knockout (KO) mice have essentially “normal” endocrine signaling, Ames dwarf, Snell dwarf, and GHR-KO mice have primary and secondary endocrine abnormalities in the growth hormone (GH) and insulin/IGF-1 pathway (Bartke et al. 2011; Coschigano et al. 2003). These different types of long-lived dwarf mice are similar in various metabolic parameters, such as suppressed levels of circulating IGF-1, insulin, and glucose (Berryman et al. 2008; Brown-Borg et al. 1996; Bartke et al. 2005). In PAPP-A KO mice, circulating levels of IGF-1 are not different when compared to normal littermates (Conover and Bale 2007). However, bioactive IGF-1 is decreased, highlighting a role of IGF-1 levels in relation to lifespan, as already established in various types of dwarf mice with low growth hormone signaling (Berryman et al. 2008; Brown-Borg et al. 1996; Bartke et al. 2005) and suggested by recent findings in humans (Milman et al. 2014).

Previous studies reported that type 2 diabetic patients with and without hypercholesterolemia have higher levels of PAPP-A in serum (Pellitero et al. 2009), suggesting that glycemic control is associated with PAPP-A expression. Longevity may be altered by metabolic stressors, such as high-energy diets, which are known to alter and possibly impair the IGF-1/insulin pathway. This metabolic impairment is now recognized as an important component of obesity-linked inflammatory diseases including insulin resistance, fatty liver disease, and atherosclerosis (Sethi and Hotamisligil 1999), therefore increasing the risk of metabolic diseases and reducing longevity. Combination diets such as high-fat and high-simple carbohydrate (sucrose) (HFHS) diet, which

promote obesity and induce inflammation, are associated with metabolic syndrome. However, the diet's effects on proteases, including PAPP-A, and its further regulation of the insulin/IGF-1 system remain to be elucidated. Thus, we investigated the effects of the *papp-a* knockout on the effects of a high-fat/high-sucrose diet at an age when excessive dietary lipids and carbohydrate consumption promote considerable metabolic dysfunction that may be associated to aging in homozygous normal (+/+) compared to the homozygous knockout (-/-) littermates.

Material and methods

Animals and diet

Heterozygous PAPP-A^{+/-} mice were generously provided by Dr. C. Conover (Division of Endocrinology, Metabolism and Nutrition, Endocrine Research Unit, Mayo Clinic, Rochester, MN, USA). These mice were generated on a mixed C57BL/6 and 129 background and are susceptible to diet-induced obesity. To establish our PAPP-A colony, PAPP-A^{+/-} male and female mice were mated. Progeny consists PAPP-A^{-/-} (dwarf), PAPP-A^{+/+}, and PAPP-A^{+/-} (the latter two, which are phenotypically indistinguishable, were used as normal controls). Animals were entered in the study at 12–14 months of age and were provided ad libitum with nutritionally balanced experimental diets. In experiment 1, four experimental groups consisted of PAPP-A KO (*n*=10) and normal littermate (*n*=10) females fed a low-fat, low-sucrose diet (LFLS: D12328 Research Diets, New Brunswick, NJ) and PAPP-A KO (*n*=10) and normal littermate (*n*=10) females fed a HFHS diet (D12331 Research Diets, New Brunswick, NJ) for 12 weeks (See Supplementary Table 1 for detailed nutrient ingredients). In experiment 2, two experimental groups consisted of PAPP-A KO (*n*=4) and normal littermate (*n*=3) fed a standard chow diet (STD; 5001* Lab Diet, St. Louis, MO) until 12 months of age and subsequently switched to HFHS diet (D12331 Research Diets, New Brunswick, NJ) for 10 weeks for calorimetric analysis. Food intake was measured weekly for 10 weeks. Average daily feed intake was expressed as food (g)/day/bw (g). The animals were housed under temperature- and light-controlled conditions (20–23 °C, 12-h light/12-h dark cycle). All animal protocols for this study were approved by the Southern Illinois University

School of Medicine Laboratory Animal Care and Use Committee.

Age category classification

PAPP-A KO mice and normal littermate mice were middle-aged (Conover and Bale 2007, Conover et al. 2008) in all experiments in order to address gerontological queries regarding metabolic function and immune response. Thus, we categorized middle age as the period between when approximately 90 % of the control subjects are still alive and median survivorship and old age as the period between median survivorship and when approximately 10 % of the subjects are alive (Arum et al. 2014).

Body weight and fat pad weight

Body weights were recorded weekly. White adipose tissue (WAT) and subcutaneous (inguinal) visceral (retroperitoneal) and intrascapular brown adipose tissue (iBAT) were removed and weighed at tissue harvest. Normalized adipose tissues weights were calculated by the following: final weight of fat pad/final whole body weight×100.

Fasted glucose

During the 12-week feeding phase of the experiment, mice were fasted overnight for 16 h on a weekly basis. Blood was collected by cutting the tip of the tail, and fasted glucose was measured by a glucometer (WaveSense, Agamatrix Inc, Salem, NH).

Glucose tolerance testing

Mice were fasted for 16 h, blood glucose was measured, and glucose at a dose of 1 g /kg of body weight was injected i.p. (time 0). Sequential glucose measurements were taken by glucometer at 15, 30, 45, 60, and 120 min (Menon et al. 2014).

Insulin sensitivity testing

Mice were fasted by removing food 1 h before testing, and insulin at a dose of 0.1 international units (IU) was injected i.p. (time 0). Sequential glucose measurements were taken by glucometer at 15, 30, 45, 60, and 120 min (Menon et al. 2014).

PAPP-A gene expression

Hepatic levels of mouse PAPP-A mRNA were measured by real-time polymerase chain reaction (PCR) using β -2 microglobulin (β 2M) as a housekeeping gene. The real-time PCR was performed using the Smart Cycler instrument (Cepheid, Sunnyvale, CA) with iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA). Each reaction contained 12.5 μ l of iQ SYBR Green Supermix, 0.4 μ M each of mouse PAPP-A, 5'-CGGCTTCCTGTTGGAC-3' forward and 5'-CAATCTGTTGCCAGCTCA-3' reverse primers (Conover et al. 2010), and 2 μ l of cDNA (after dilution 3 H₂O:1 cDNA mix). The three steps of the PCR included denaturation at 94 °C for 2 min, annealing at 62 °C for 30 s with fluorescence reading, and extension at 72 °C for 30 s. In addition, a melting curve was used to evaluate the potential of nonspecific products. All samples were analyzed in a single reaction for PAPP-A gene. The comparative CT method was used to determine fold differences between samples. The comparative CT method determines the amount of target, normalized to an endogenous reference (β 2M) and relative to a calibrator ($2^{-\Delta\Delta C_t}$) (Menon et al. 2014).

Measurements of insulin, IGF-1, adiponectin, leptin, and PAPP-A

After 12 weeks of feeding the indicated diets, mice were fasted for 16 h for tissue harvest. Following isoflurane (Butler Animal Health Supply, Dublin, Ohio) anesthesia, mice were bled by cardiac puncture; blood was mixed with EDTA, followed by centrifugation at 10,000 $\times$ g for 15 min at 4 °C for plasma collection. Plasma insulin levels were determined using Ultra-Sensitive Rat Insulin Enzyme-Linked Immunosorbent Assay (ELISA) kit (Crystal Chem Inc., Downers Grove, IL). Plasma IGF-1 levels were determined using a Rat/Mouse IGF-1 ELISA kit (Immunodiagnostic Systems, Adelaide, Australia). Plasma adiponectin levels were determined using a Mouse Adiponectin ELISA Kit (Linco Research, St Charles, MO). Plasma leptin levels were determined using a Mouse Leptin ELISA Kit (Crystal Chem Inc., Downers Grove, IL). PAPP-A levels were determined using a Mouse PAPP-A-ELISA Kit (BG-MUS11883: Nova Tein Bio, Inc., Cambridge, MA). Protocols for

all assays were performed according to the manufacturers' manuals.

Plasma cytokine measurements

The cytokine levels were determined using a Q-Plex™ Mouse Cytokine Chemiluminescent array (4-plex, Quansys Biosciences, Logan, UT) according to the manufacturer's instructions. Briefly, the plasma and antigen standards were diluted; 50 μ l of standards and samples were pipetted into a 96-well plate and incubated for 1 h at room temperature. After the substrates were added, images were acquired using a FUJI LAS4000 camera and analyzed with Quansys Q-View™ Software.

Indirect calorimetry

For indirect calorimetry, we used adult (12–14 months of age) PAPP-A KO mice ($n=4$) and normal littermates ($n=3$). Indirect calorimetry was performed using the AccuScan Instruments, Inc. PhysioScan Metabolic System (Columbus, Ohio). This system utilizes zirconia and infrared sensors to monitor oxygen (O₂) and carbon dioxide (CO₂), respectively, inside of respiratory chambers in which individual mice are tested. All comparisons are based on animals studied simultaneously in an effort to minimize the effect of environmental variations and calibration on data. After 24 h acclimation period, mice were monitored in the metabolic chambers for 24 h with ad libitum access to assigned diet (STD or HFHS) and water. Gas samples were collected and analyzed every 10 min per animal. Output parameters include oxygen consumption (VO₂—mL/kg/min) and respiratory quotient (RQ—VCO₂/VO₂).

Statistical analysis

Analyses were performed by two-way ANOVA test and Student's *t* test within groups when justified. Repeated measures ANOVA was used to determine the interaction and diet effects for insulin tolerance test (ITT) and glucose tolerance test (GTT). All statistics and graphs were done using Prism 5 (GraphPad Software, San Diego, CA, USA). Alpha was set to 0.05. Values are reported as mean $\pm$ standard error of the mean (SEM) throughout the figures.

Results

Experiment 1

PAPP-A KO mice accumulate less visceral fat when fed high-fat, high-sucrose diet

Subjecting animals to HFHS feeding significantly increased absolute body weight gain in both normal (N) ($P=0.0001$) and PAPP-A KO ($P=0.001$) mice when compared to the corresponding controls fed an LFLS diet (Fig. 1a). However, the HFHS diet had greater effect on body weight gain in PAPP-A KO mice, leading to a 53 % weight increase during the treatment, compared to only a 20 % increase in N mice (Fig. 1b). Subcutaneous WAT and retroperitoneal WAT significantly increased in both N mice and PAPP-A KO mice fed HFHS diet when compared to corresponding mice fed LFLS diet ($P<0.05$; Fig. 1c, d). Interscapular brown adipose tissue (iBAT) was significantly reduced in both N and PAPP-A KO mice fed HFHS diet compared to corresponding controls fed LFLS diet ($P\leq 0.02$; Fig. 1e). Yet, iBAT was significantly higher in PAPP-A KO mice fed HFHS diet compared to N mice fed HFHS diet ($P=0.04$; Fig. 1e). In addition, iBAT was numerically higher in PAPP-A KO mice fed LFLS compared to N mice fed LFLS diet.

PAPP-KO mice are resistant to the induction of metabolic dysfunction by HFHS diet

Glucose tolerance testing determined that PAPP-A KO mice fed HFHS diet for 6 weeks were significantly more glucose tolerant than N mice fed the same diet at 30 min ($P=0.03$; Fig. 2a). Significantly, during the tenth week of the feeding phase, lower clearance of glucose was observed in N mice compared to PAPP-A KO mice at 30 ($P=0.02$), 45 ($P=0.04$), and 60 min ($P=0.006$) after glucose injection (Fig. 2b). These data are comparable to recent observations that PAPP-A KO mice fed a high-fat diet for 20 weeks appeared to have enhanced glucose tolerance; however, this apparent effect was not statistically significant (Conover et al. 2013). Furthermore, PAPP-A KO mice fed an HFHS diet showed similar glucose tolerance as PAPP-A KO mice and N mice fed a LFLS diet. No differences were observed in PAPP-A KO mice compared to N mice fed LFLS diet. Insulin tolerance test performed at 6 weeks of treatment did not show any effects of either genotype of diet (Fig. 2c). However, after 10 weeks of feeding an HFHS diet, PAPP-A KO mice showed enhanced insulin sensitivity when compared to N mice fed HFHS ($P=0.02$; Fig. 2d) as determined by area under

Fig. 1 Visceral fat is increased in PAPP-A KO mice fed an HFHS diet. PAPP-A KO and normal littermates consumed either an LFLS or HFHS diet for 12 weeks. Weekly body weights were recorded during the feeding phase. **a** Absolute weight gain. **b**

Normalized body weight gain. **c** Normalized percentage gains in subcutaneous adipose tissue (AD), retroperitoneal AD, and brown AD. All values are presented as mean. The error bars represent the SEM. * $P<0.05$

curve ($P=0.001$) (Supplementary Fig. 1). Further, PAPP-KO mice fed an HFHS diet seemed to be more insulin sensitive when compared to N and PAPP-KO mice fed an LFLS diet; however, this trend was not statistically significant (Fig. 2d).

Fasted blood glucose levels increased in both PAPP-A KO ($P=0.001$) and N ($P=0.03$) mice fed an HFHS diet compared to the corresponding controls fed with LFLS diet for 12 weeks (Fig. 2e). Fasted insulin levels were higher in N mice fed HFHS than in PAPP-A KO mice fed either diet ($P=0.001$; Fig. 2f). Although insulin levels numerically increased in N mice fed an HFHS diet, this apparent effect was not statistically significant compared to N mice fed an LFLS diet (Fig. 2f).

Surprisingly, fasted insulin levels in PAPP-A KO mice were not altered by the HFHS diet (Fig. 2f). Glucose homeostasis involves whole-body glucose tolerance and insulin sensitivity, and we questioned whether alterations in IGF-1 might contribute to the effects of HFHS diets on these parameters. Insulin-like growth factor-1 levels were lower in PAPP-A KO mice fed an HFHS diet when compared to N mice fed an HFHS diet ($P=0.02$; Fig. 2g). Yet, there was a significant increase in IGF-1 levels in PAPP-A KO mice fed an HFHS diet compared to PAPP-A KO and N mice fed an LFLS diet ($P=0.002$; Fig. 2g). Furthermore, HFHS-fed N mice had increased IGF-1 levels when compared to N mice fed an LFLS diet ($P=0.04$; Fig. 2g).

Fig. 2 Long-lived PAPP-A KO mice are resistant to metabolic dysfunction induced by an HFHS diet. PAPP-A KO and normal littermates consumed either an LFLS or HFHS diet for 12 weeks; GGT were performed during week 6 and week 10 of the study. **a, b** Glucose tolerance test (GTT) at 6 and 10 weeks. Sixteen-hour-fasted mice underwent GTT by i.p. injection with 1 g glucose per

kg of BW. **c, d** Insulin tolerance test (ITT) at 6 and 10 weeks. Mice were injected i.p. with 0.1 IU porcine insulin per kilogram of BW. ITT data expressed as percentage of initial blood glucose concentration. **e** Average blood glucose levels. **f** Fasted insulin levels. **g** Circulating IGF-1 levels. All values are presented as mean. The error bars represent the SEM. * $P<0.05$, ** $P<0.001$

PAPP-A KO mice are protected from pro-inflammatory effects of HFHS

Previous studies have shown that PAPP-A is regulated by pro-inflammatory cytokines (Pellitero et al. 2009). These cytokines may be responsive to injury, but whether a calorie-dense diet increases cytokines that promote the expression of PAPP-A was unknown. Our immunosorbent analysis indicated that PAPP-A KO mice fed an HFHS diet had lower circulating levels of mouse interleukin-2 (mIL-2), mIL-6, and mTNF- α pro-inflammatory cytokines when compared to N mice fed an HFHS diet (Fig. 3a–c). mIL-2 levels significantly increased in N mice fed an HFHS diet when compared to N mice fed an LFLS diet and PAPP-A KO mice fed either diet ($P=0.0001$; Fig. 3a). mIL-6 levels were increased in N mice fed an HFHS diet when compared to N mice fed an LFLS diet ($P=0.004$; Fig. 3b). Furthermore, mIL-6 levels remained low in PAPP-A KO mice fed an HFHS diet when compared to N mice fed an HFHS diet ($P=0.006$; Fig. 3b). Although mIL-6 levels seemed to be elevated in PAPP-A KO mice fed an HFHS diet, this trend was not statistically significant (Fig. 3b). mTNF- α levels were increased in N mice fed an HFHS diet when compared to N mice fed an LFLS diet ($P=0.05$; Fig. 3c). In addition, PAPP-A KO mice fed an HFHS diet retained low levels of TNF- α compared to N mice fed an HFHS diet ($P=0.001$) and did not significantly differ from PAPP-A KO mice fed an LFLS diet (Fig. 3c).

Anti-inflammatory cytokines may function as intracellular protection by reducing IL-6 and rescuing insulin signaling. PAPP-A KO mice fed an HFHS diet had higher levels of anti-inflammatory cytokines mIL-4 and adiponectin when compared to N mice fed the same diet (Fig. 3d, e). HFHS feeding in N mice numerically decreased mIL-4 levels compared to N mice fed an LFLS diet. In contrast, in PAPP-A KO mice fed an HFHS diet, mIL-4 levels were elevated ($P=0.01$) compared to N mice fed an HFHS diet and not statistically different from the levels measured in PAPP-A KO mice fed an LFLS diet. Adiponectin levels were drastically decreased in N mice fed an HFHS diet compared to N mice fed an LFLS diet and PAPP-A KO mice fed either diet ($P=0.0001$; Fig. 3e). Furthermore, adiponectin levels were higher in PAPP-A KO mice fed an LFLS diet compared to N mice fed an LFLS diet ($P=0.01$; Fig. 3e); strikingly, PAPP-A KO mice fed an HFHS diet did not differ in this regard from mice fed an LFLS diet.

High-fat, high-sucrose diet increased PAPP-A levels in normal littermates

We questioned whether there is a direct effect of HFHS diet on the expression and levels of pro-inflammatory cytokines and expression of PAPP-A levels in mice. We have shown that HFHS diet influences the expression of pro-inflammatory cytokines that are a likely contributor

Fig. 3 Anti-inflammatory cytokine levels are increased in PAPP-A KO mice fed an HFHS diet. Mice were fasted for 16 h, and collected plasma was used for assays. **a** Mouse interleukin 2 (mIL-

2). **b** mIL-6. **c** mTNF- α . **d** Anti-inflammatory mIL-4. **e** Adiponectin. All values are presented as mean. The error bars represent the SEM. * $P<0.05$, ** $P<0.001$, *** $P<0.0001$

to the expression of PAPP-A. As determined by q-PCR, IL-6 mRNA was increased in N mice fed an HFHS diet when compared to N mice fed an LFLS diet ($P=0.002$; Fig. 4a). Expression of PAPP-A mRNA was significantly increased in hepatic tissue of N mice fed an HFHS diet when compared to N mice fed LFLS ($P=0.005$; Fig. 4b). Results of an immunosorbent assay indicated that PAPP-A protein levels were also increased by an HFHS diet in N mice ($P=0.05$; Fig. 4c).

Fig. 4 Normal mice fed an HFHS diet have an increased level of PAPP-A protease. **a** Relative gene expression of hepatic IL-6 in normal mice fed either an LFLS ($n=6$) or HFHS ($n=6$) diet. **b** Relative gene expression of hepatic PAPP-A in normal mice fed either an LFLS ($n=6$) or HFHS ($n=6$) diet. **c** Circulating PAPP-A protein levels in mice fed either an LFLD ($n=6$) or HFHS ($n=6$). All values are presented as mean. The error bars represent the SEM. * $P<0.05$, ** $P<0.001$

Experiment 2

Indirect calorimetry

PAPP-A KO mice are sensitive to weight gain in response to HFHS feeding but are resistant to the metabolic dysfunction effects of HFHS as revealed in ITT and GTT. Therefore, we questioned whether their metabolic phenotype would clarify their resistance to the

metabolic stressors associated with HFHS diet. We thus utilized indirect calorimetry analysis in a subset of mice to monitor respiratory quotient, oxygen consumption, and carbon dioxide release in PAPP-A KO mice and N mice fed STD. We then switched these *same* mice (fed STD) to HFHS diet for 10 weeks, thus allowing observations of possible metabolic switch within same animals.

Body weight and feed intake

As expected, PAPP-A KO mice and N mice gain weight respectively to their initial body weight during the 10-week phase of HFHS diet feeding ($P=0.0001$; Fig. 5a). Furthermore, food intake in PAPP-A KO mice fed HFHS diet was significantly higher compared to N mice fed HFHS diet ($P=0.0001$; Fig. 5b). Food intake is predominately regulated by leptin. Leptin is a satiety hormone, abundantly synthesized and secreted by adipocytes, and plasma levels are in proportion to adiposity (Sahu et al. 1993); it regulates food intake and energy expenditure to maintain body fat storage (Klok et al. 2007). We observed that PAPP-A KO mice fed HFHS diet had significantly lower levels of circulating leptin compared to N mice fed HFHS diet ($P=0.0001$; Fig. 5c).

Oxygen consumption Oxygen consumption, expressed as VO_2 , is the volume of oxygen consumed by an organism over a given period of time (Westbrook et al. 2009). VO_2 measurements illustrated the expected diurnal rhythm of increased VO_2 consumption during the dark period, indicating increased movement and feeding of all mice. We first investigated whether oxygen consumption differed in PAPP-A KO mice and N mice fed STD diet. We then evaluated whether oxygen consumption would be altered in either PAPP-A KO mice or N mice after being switched from STD diet to HFHS diet for 10 weeks. Throughout the 24-h measurement period, our indirect calorimetry data showed that PAPP-A KO mice consume more oxygen per gram body weight when compared to N mice when fed STD diet ($P=0.001$; Fig. 5d). Remarkably, after 10 weeks of consuming HFHS diet, PAPP-A KO mice retain the ability to consume significantly more oxygen when compared to their N counterparts fed the same HFHS diet ($P=$

0.001; Fig. 5e). Further, PAPP-A KO mice fed STD release significantly more carbon dioxide when compared to N mice fed STD (Supplemental Fig. 2). Interestingly, PAPP-A KO mice switched from STD to HFHS maintain the ability to release more carbon dioxide than N mice fed HFHS diet (Supplemental Fig. 3).

Respiratory quotient The RQ value is calculated using the equation carbon dioxide (CO_2) eliminated/oxygen (O_2) consumed (Dudley 1977). The ratio of the amount of CO_2 that is eliminated from the body to the O_2 that is consumed reflects the use of lipids or carbohydrates as an energy source. The values of RQ for organisms in metabolic balance usually range from 1.0, which represents the value expected for pure carbohydrate oxidation, to approximately 0.7, which represents value expected for pure fat oxidation (Lusk and Riche 1912; Lusk 1928). All mice illustrated the expected diurnal rhythm of increased RQ during the dark period, indicating increased movement and feeding. There was no difference in RQ for the 12-h light or 12-h dark cycle period during 24-h measurement in PAPP-A KO mice fed STD when compared to N mice fed STD (Fig. 5c). However after 10 weeks of HFHS diet, RQ values for PAPP-A KO mice significantly lower during the 12-h light ($P=0.01$) and 12-h ($P=0.001$) dark cycle period when compared to N mice fed HFHS during the 24-h measurement (Fig. 5d). These data indicate that PAPP-A KO mice fed HFHS diet are able to rely on fat as their primary source of energy more so than their littermate controls.

Discussion

Three major strong associations involving gene expression and mechanisms of longevity in mammals are confirmed in the current study: the relationship of adiponectin to insulin sensitivity; the relationship of diet and pro-inflammatory cytokines to lifespan; and the suggestion of metabolic flexibility as protection from calorie-dense diets. The relationship of insulin/IGF-1 signaling to stress resistance and longevity is further supported by the observation that PAPP-A KO females are more resistant to the induction of diabetic nephropathy by streptozotocin treatment when compared to wild-type mice (Mader et al. 2013).

Fig. 5 PAPP-A KO mice fed an HFHS diet have increased VO₂ and decreased RQ. PAPP-A KO mice ($n=4$) and normal littermates ($n=3$) fed STD were placed in the metabolic chambers to measure VO₂ and RQ. Following initial metabolic measurements, PAPP-A KO mice ($n=4$) and normal littermates ($n=3$) fed STD for 12 months and sequentially switched to HFHS for 10 weeks. Body weights were recorded during the feeding phase, mice were placed

in indirect calorimetry chambers, and at the terminal phase plasma was collected for leptin assay. **a** Absolute weight gain. **b** Average food intake. **c** Circulating leptin levels. **d** VO₂ in PAPP-A KO mice and normal mice fed STD. **e** VO₂ in PAPP-A KO mice and normal mice fed HFHS. **f** RQ in PAPP-A KO mice and normal mice fed STD. **g** RQ in PAPP-A KO mice and normal mice fed HFHS

Consumption of calorie-dense diets without physical activity increases fat accumulation primarily in WAT. Visceral WAT (VIS) influences whole-body glucose homeostasis, and excessive VAT accumulation is associated with metabolic syndrome, including insulin resistance (Rasouli et al. 2007). Total weight gain of VIS was increased in both PAPP-A KO mice and N littermates fed an HFHS diet, and this gain was increased proportionally to their initial body weight. Yet, PAPP-A KO mice fed HFHS diet gained less VIS compared to total VIS gain in N mice fed HFHS diet suggesting that PAPP-A deficiency alters VIS accumulation. The degree of fat storage is dependent on lipolysis, which results in fatty acid release from the cell and consequently a decrease of adipocyte size. Conover et al. showed that PAPP-A KO mice fed high-fat diet (HFD) for

20 weeks restrained enlargement of visceral adipocyte size and cell number compared to normal mice fed HFD (Conover et al. 2013). In addition, other studies have shown that PAPP-A expression significantly decreased expression of genes associated to lipid metabolism (i.e., LXR α pathway) at transcriptional and translational levels in pathogenesis of atherosclerosis (Tang et al. 2012). These data suggest that the deletion of *papp-a* is associated to smaller adipocyte size and cell number in VIS-WAT, even during consumption of calorie dense diets, and promotes lipid metabolism in adipocytes.

Extended longevity of PAPP-A KO mice reflects reduced levels of free bioactive IGF-1. Here, we show that an HFHS diet increased IGF-1 levels in PAPP-A KO mice when compared to PAPP-A KO fed LFLS, yet it did not impair glucose homeostasis as measured by

ITT and GTT in these animals. We observed that PAPP-A KO mice fed an HFHS diet have improved glucose assimilation during GTT and are more insulin sensitive, despite increased adiposity, compared to their N littermates fed an HFHS diet. This phenotype resembles other long-lived mice, namely Ames dwarf and GHR-KO mice which have increased adiposity as a result of either deleted or diminished growth hormone signaling. Although Ames dwarf mice have a “chubby” phenotype, they are extremely insulin sensitive, have lower levels of insulin, as well as increased lifespan compared to N littermates when fed a standard chow and calorie-restricted diet (Brown-Borg et al. 1996; Bartke et al. 2011). Recently, we have observed that Ames dwarf mice fed a HFD (60 % fat) for 12 weeks increase in adiposity yet remain insulin sensitive and have low levels of insulin (*unpublished data*). Insulin levels were lower in PAPP-A KO fed an HFHS diet when compared to normal littermates fed HFHS and did not differ from those measured in PAPP-A KO fed LFLS. This was surprising in view of the documented effects of calorie-dense diets in the etiology of metabolic syndrome, including hyperinsulinemia (Coppack 2005). We suspect that high IGF-1 levels in PAPP-A KO mice fed HFHS may have contributed to physiological compensation to maintain glucose homeostasis in these mice (Blundell and Humbell 1980). These data indicate that PAPP-A KO mice are protected from metabolic dysfunction induced by HFHS feeding, taking into account that this diet is a combination of high-calorie nutrients.

Prolonged consumption of diets high in fat induces obesity and promotes local and chronic systemic inflammation with consequent changes impairing insulin signaling in mice (Wiedemann et al. 2013) and humans (Seith et al. 1999). Pro-inflammatory cytokines such as IL-6 and TNF- α are elevated in obesity and insulin resistance. IL-6 is elevated by high insulin levels and TNF- α in plasma and WAT, and it impacts insulin signaling by switching tyrosine to serine phosphorylation of the insulin receptor substrate-1 (IRS-1) (Fasshauer et al. 2003). Furthermore, IL-2 induces low-grade inflammation through the activation of adipocytes and type 1 T-helper (Th1) cell migration promoting insulin resistance (Wagner et al. 2013; Olefsky and Glass 2010; Gordon 2003). Our data show that PAPP-A KO female mice fed a combined HFHS diet have lower levels of inflammatory cytokines IL-2, IL-6, and TNF- α when compared to their N littermates fed an HFHS diet, thus escaping “obesity-linked inflammatory

diseases.” Furthermore, PAPP-A KO mice fed an HFHS diet have higher levels of anti-inflammatory cytokines IL-4 and adiponectin.

Interleukin 4 represents an anti-inflammatory response to stress conditions such as wound healing and tissue remodeling by enhancing the mannose receptor, the IL-1 receptor antagonist that elicits a pro-inflammatory response (Stein et al. 1992). The intracellular mechanism by which IL-4 functions is partially mediated by signal transducer and activator of transcription 6 (STAT6) (Ohmori et al. 1996). Activated transcription factors phosphorylate Janus Kinase 3 (JAK 3) at a tyrosine residue (Tyr641) after IL-4 binds to the IL-4 receptor and induces IL-4-related genes, including adiponectin (Takeda et al. 1996; Nelms et al. 1999; Mandal et al. 2011).

Adiponectin is an adipocyte-derived cytokine that has well-characterized anti-inflammatory effects, including suppression of macrophage differentiation and migration (Yokota et al. 2000), chemokine production, and T cell migration (Okamoto et al. 2008). Furthermore, adiponectin levels correlate with the delayed aging and enhanced insulin sensitivity. For example, an increase in adiponectin has been well established in long-lived mice such as Ames dwarf (Wang et al. 2006), Snell dwarf (Brooks et al. 2007), and GHR-KO (Berryman et al. 2011) mice, and adiponectin is more abundant in plasma of human subjects without metabolic syndrome (Bik and Baranowska 2009; Coppack 2005). Here, we show that HFHS diet suppresses adiponectin levels in N mice, but PAPP-A KO mice are completely protected from this effect. Furthermore, HFHS-fed N littermates had lower levels of adiponectin when compared to LFLS-fed N littermates. To support our findings, recent studies show that PAPP-A KO mice fed HFD for 10 weeks had higher mRNA level of adiponectin and lower mRNA levels of TNF- α in perigonadal adipose tissue (Conover et al. 2013). As in reference to PAPP-A KO mice having smaller adipocyte size, it can be assumed that smaller adipocyte size and increased adiponectin possibly protect PAPP-A KO mice fed HFHS from metabolic dysfunction.

Pro-inflammatory cytokines (PICs) such as IL-6 and TNF- α mediate the expression of PAPP-A protease in cell culture (Boldt and Conover 2007). Furthermore, studies suggest that glycemic control is associated with PAPP-A expression in human subjects (Pellittero et al. 2009). Thus, we questioned whether PAPP-A protease levels would be altered by an HFHS diet in N littermates.

We show that the N littermates fed an HFHS diet had higher PAPP-A protease mRNA and protein levels than the N littermates fed an LFLS diet. These data strongly support the results of in vitro studies (Boldt and Conover 2007) that associate PAPP-A protease expression with PICs. Importantly, we observed the expected detrimental outcome in the N littermates fed an HFHS diet, in which PICs are increased and PAPP-A protease levels are higher when compared to N littermates fed an LFLS diet.

Lastly, we investigated the metabolic phenotype of PAPP-A KO mice and N mice fed STD by indirect calorimetry. PAPP-A KO mice fed STD consume more oxygen compared to normal mice fed STD, while respiratory quotient did not differ in PAPP-KO mice compared to N mice fed STD. However, at 10 weeks of feeding HFHS diet, significantly increased oxygen consumption (VO_2) was observed in PAPP-A KO mice fed HFHS diet compared to normal mice fed the same diet. These data may support the theory that increased oxygen consumption (VO_2) has a positive relationship to longevity and decreases the production of reactive oxygen species (ROS) in mitochondria. An increase in VO_2 per gram body weight in smaller mice is also associated with a significant reduction in RQ (Westbrook et al. 2009). RQ provides an approximation of the use of metabolic fuels to generate energy with an RQ of 1.0 representing use of carbohydrates and RQ of 0.7 representing use of fats (Lusk 1912, 1928). β -oxidation of fatty acids also known as “fat burning” represents an increased dependence on fats to satisfy energy needs and has been associated with improved metabolic homeostasis and extended longevity in humans and mice (Westbrook et al. 2009; Zurlo et al. 1990; Seidell et al. 1992; Solaini et al. 2010). We observed that RQ was significantly lower in PAPP-A KO mice fed HFHS diet compared to N mice fed HFHS diet. In addition, a decrease in iBAT in PAPP-A KO mice fed HFHS might be related “browning” of WAT inducing thermogenesis in this adipose tissue compartment. We may suggest that PAPP-A KO mice fed HFHS diet utilize fatty acids as a major source of fuel through β -oxidation.

We conclude that PAPP-A KO female mice are resistant to the detrimental metabolic effects of HFHS, apparently due to the protection provided by the increase of anti-inflammatory cytokines and suppression of pro-inflammatory cytokines and a remarkably metabolic flexible phenotype. Furthermore, the expression of the PAPP-A protease has a role in glucose homeostasis

as determined by increased levels of pro-inflammatory cytokines and impaired insulin signaling observed in N littermates fed HFHS.

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Conflict of interest The authors declare that they have no conflict of interest.

References

- Arum O, Saleh JK, Boparai RK, Kopchick JJ, Khardori RK, Bartke A (2014) Preservation of blood glucose homeostasis in slow-senescent somatotrophism-deficient mice subjected to intermittent fasting begun at middle or old age. *Age (Dordr)* 36(3):9651
- Bartke A (2003) Can growth hormone (GH) accelerate aging? Evidence from GH-transgenic mice. *Neuroendocrinology* 78(4):210–216
- Bartke A, Wright JC, Mattison JA, Ingram DK, Miller RA, Roth GS (2011) Extending the lifespan of long-lived mice. *Nature* 414(6862):412
- Berryman DE, Christiansen JS, Johannsson G, Thorner MO, Kopchick JJ (2008) Role of the GH/IGF-I axis in lifespan and healthspan: lessons from animal models. *Growth Horm IGF Res* 18(6):455–471
- Berryman DE, List EO, Sackmann-Sala L, Lubbers E, Munn R, Kopchick JJ (2011) Growth hormone and adipose tissue: beyond the adipocyte. *Growth Horm IGF Res* 21:113–123
- Bik W, Baranowska B (2009) Adiponectin—a predictor of higher mortality in cardiovascular disease or a factor contributing to longer life? *Neuro Endocrinol Lett* 30:180–184
- Blundell TL, Humbel RE (1980) Hormone families: pancreatic hormones and homologous growth factors. *Nature* 30(287):781–787
- Boldt HB, Conover CA (2007) Pregnancy-associated plasma protein-A (PAPP-A): a local regulator of IGF bioavailability through cleavage of IGFBPs. *Growth Horm IGF Res* 17:10–18
- Braun S, Bitton-Worms K, LeRoith D (2011) Review: the link between the metabolic syndrome and cancer. *Int J Biol Sci* 7(7):1003–1015
- Brooks NL, Trent CM, Raetzsch CF, Flurkey K, Boysen G, Perfetti MT, Jeong YC, Klebanov S, Patel KB, Khodush VR, Kupper LL, Carling D, Swenberg JA, Harrison DE, Combs TP (2007) Low utilization of circulating glucose after food withdrawal in Snell dwarf mice. *J Biol Chem* 282(48):35069–35077
- Brown-Borg HM, Borg KE, Meliska CJ, Bartke A (1996) Dwarf mice and the ageing process. *Nature* 384:33

- Combs GF Jr (2008) The vitamins: fundamental aspects in nutrition and health, 3rd edn. Elsevier Academic Press, New York, p 66
- Conover CA, Bale LK (2007) Loss of pregnancy-associated plasma protein A extends lifespan in mice. *Aging Cell* 6:727–729
- Conover CA, Faessen GF, Ilg KE, Chandrasekher YA, Christiansen M, Overgaard MT, Oxvig C, Giudice LC (2001) Pregnancy-associated plasma protein-a is the insulin-like growth factor binding protein-4 protease secreted by human ovarian granulosa cells and is a marker of dominant follicle selection and the corpus luteum. *Endocrinology* 142:2155
- Conover CA, Mason MA, Levine JA, Novak CM (2008) Metabolic consequences of pregnancy-associated plasma protein-A deficiency in mice: exploring possible relationship to the longevity phenotype. *J Endocrinol* 198(3):599–605
- Conover CA, Mason MA, Bale LK, Harrington SC, Nyegaard M, Oxvig C, Overgaard MT (2010) Transgenic overexpression of pregnancy-associated plasma protein-A in murine arterial smooth muscle accelerates atherosclerotic lesion development. *Am J Physiol Heart Circ Physiol* 299(2):H284–H291
- Conover CA, Harstad SL, Tchkonja T, Kirkland JL (2013) Preferential impact of pregnancy-associated plasma protein-A deficiency on visceral fat in mice on high-fat diet. *Am J Physiol Endocrinol Metab* 305(9):E1145–E1153
- Coppack SW (2005) Adipose tissue changes in obesity. *Biochem Soc Trans* 33(5):1049–1052
- Coschigano KT, Holland AN, Riders ME, List EO, Flyvbjerg A, Kopchick (2003) Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin and IGF-1 levels and increased lifespan. *Endocrinology* 144:3799–3810
- Dudley BAC (1977) Mathematical and biological interrelations. Wiley, New York
- Fasshauer M, Klein J, Lossner U, Paschke R (2003) Interleukin (IL)-6 mRNA expression is stimulated by insulin, isoproterenol, tumour necrosis factor alpha, growth hormone, and IL-6 in 3T3-L1 adipocytes. *Horm Metab Res* 35:147–152
- Flurkey K, Papaconstantinou J, Harrison DE (2002) The Snell dwarf mutation *Pit1* (*dw*) can increase life span in mice. *Mech Ageing Dev* 123(2–3):121–130
- Gordon S (2003) Alternative activation of macrophages. *Nat Rev Immunol* 3(1):23–35
- Klok MD, Jakobsdottir S, Drent ML (2007) The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review. *Obes Rev* 8:21–34
- Lusk G (1928) The elements of the science of nutrition, 4th edn. B. Saunders Co, Philadelphia
- Lusk G, Riche JA (1912) Animal calorimetry; metabolism after the ingestion of dextrose and fat. *J Biol Chem* 13:27–47
- Mader JR, Resch ZT, McLean G, Mikkelsen J, Oxvig C, Marler RL, Conover CA (2013) Mice deficient in PAPP-A show resistance to the development of diabetic nephropathy. *J Endocrinol* 219(1):51–58
- Mandal P, Pratt BT, Barnes M, McMullen MR, Nagy LE (2011) Molecular mechanism for adiponectin-dependent M2 macrophage polarization: link between the metabolic and innate immune activity of full-length adiponectin. *J Biol Chem* 286(15):13460–13469
- Menon V, Zhi X, Hossain T, Bartke A, Spong A, Gesing A, Masternak MM (2014) The contribution of visceral fat to improved insulin signaling in Ames dwarf mice. *Aging Cell* 13(3):497–506
- Milman S, Atzmon G, Huffman DM, Wan J, Crandall JP, Cohen P, Barzilai N (2014) Low insulin-like growth factor-1 level predicts survival in humans with exceptional longevity. *Aging Cell* 13(4):769–771
- Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE (1999) The IL-4 receptor: signaling mechanisms and biologic functions. *Annu Rev Immunol* 17:701–738
- Ohmori Y, Smith MF Jr, Hamilton TA (1996) IL-4-induced expression of the IL-1 receptor antagonist gene is mediated by STAT6. *J Immunol* 157(5):2058–2065
- Okamoto Y, Folco EJ, Minami M, Wara AK, Feinberg MW, Sukhova GK, Colvin RA, Kihara S, Funahashi T, Luster AD, Libby P (2008) Adiponectin inhibits the production of CXC receptor 3 chemokine ligands in macrophages and reduces T-lymphocyte recruitment in atherogenesis. *Circ Res* 102:218–225
- Olefsky JM, Glass CK (2010) Macrophages, inflammation and insulin resistance. *Annu Rev Physiol* 72:219–246
- Pellitero S, Reverter JL, Granada ML, Pizarro E, Pastor MC, Tàssies D, Reverter JC, Salinas I, Sanmartí A (2009) Association of the IGF1/pregnancy-associated plasma protein-A system and adipocytokine levels with the presence and the morphology of carotid plaques in type 2 diabetes mellitus patients with stable glycaemic control. *Eur J Endocrinol* 160(6):925–932
- Rasouli N, Molavi B, Elbein SC, Kern PA (2007) Ectopic fat accumulation and metabolic syndrome. *Diabetes Obes Metab* 9(1):1–10
- Sahu S, Kalra S (1993) Neuropeptidergic regulation of feeding behavior. *Trends Endocrinol Metab* 4:217–224
- Seidell JC, Muller DC, Sorkin JD, Andres R (1992) Fasting respiratory exchange ratio and resting metabolic rate as predictors of weight gain: the Baltimore Longitudinal Study on Aging. *Int J Obes Relat Metab Disord* 16:667–674
- Sethi JK, Hotamisligil GS (1999) The role of TNF alpha in adipocyte metabolism. *Semin Cell Dev Biol* 10:19–29
- Solaini G, Baracca A, Lenaz G, Sgarbi G (2010) Hypoxia and mitochondrial oxidative metabolism. *Biochim Biophys Acta* 1797:1171–1177
- Stein M, Keshav S, Harris N, Gordon S (1992) Interleukin 4 potentially enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* 176(1):287–292
- Takeda K, Tanaka T, Shi W (1996) Essential role of Stat6 in IL-4 signaling. *Nature* 380(6575):627–630
- Tang SL, Chen WJ, Yin K, Zhao GJ, Mo ZC, Lv YC, Ouyang XP, Yu XH, Kuang HJ, Jiang ZS, Fu YC, Tang CK (2012) PAPP-A negatively regulates ABCA1, ABCG1, and SR-B1 expression by inhibiting LXRα through the IGF-1 mediated signaling pathway. *Atherosclerosis* 222 (2): 344–54
- Wagner NM, Brandhorst G, Czepluch F, Lankeit M, Eberle C, Herzberg S, Faustini V, Riggert J, Oellerich M, Hasenfuss G, Konstantinides S, Schäfer K (2013) Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. *Obesity (Silver Spring)* 21(3):461–468
- Wang Z, Al-Regaiey KA, Masternak MM, Bartke A (2006) Adipokines and lipid levels in Ames dwarf and calorie-restricted mice. *J Gerontol A Biol Sci Med Sci* 61(4):323–331

- Westbrook R, Bonkowski MS, Strader AD, Bartke A (2009) Alterations in oxygen consumption respiratory quotient and heat production in long-lived GHRKO and Ames dwarf mice, and short-lived bGH transgenic mice. *J Gerontol A Biol Sci Med Sci* 64:443–451
- Wiedemann MS, Wueest S, Item F, Schoenle EJ, Konrad D (2013) Adipose tissue inflammation contributes to short-term HFD-induced hepatic insulin resistance. *Am J Physiol Endocrinol Metab* 305(3):E388–E395
- Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, Tomiyama Y, Matsuzawa Y (2000) Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. *Blood* 96:1723–1732
- Zurlo F, Lillioja S, Esposito-Del Puente A et al (1990) Low ratio of fat to carbohydrate oxidation as predictor of weight gain: study of 24-h RQ. *Am J Physiol* 259:E650–E657