Published in final edited form as:
Exp Gerontol. 2014 September ; 0: 13–17. doi:10.1016/j.exger.2014.04.011.

Tissue-specific Changes in Pregnancy Associated Plasma Protein-A Expression with Age in Mice

Sara L. Harstad and **Cheryl A. Conover**
Division of Endocrinology, Metabolism and Nutrition, Endocrine Research Unit, Mayo Clinic, 200 First Street SW, 5-194 Joseph Building, Rochester, MN 55905

Sara L. Harstad: Harstad.Sara@mayo.edu; Cheryl A. Conover: Conover.Cheryl@mayo.edu

Abstract

Pregnancy-associated plasma protein-A (PAPP-A) is a novel zinc metalloproteinase that functions in many systems outside of pregnancy. Data in both humans and mice suggest a role for PAPP-A in aging and age-related diseases. However, our knowledge of tissue-specific PAPP-A expression and possible changes in this expression with age is limited. Thus, the aim of this study was to determine PAPP-A mRNA expression in multiple tissues with age in both male and female mice using real-time PCR. These included heart, liver, kidney, bone, fat, skeletal muscle, gonads, brain, thymus and spleen. In young mice, PAPP-A mRNA was expressed at relatively high levels in all tissues examined except for liver. The only difference in expression between males and females was seen in kidney, subcutaneous fat and gonads. The highest PAPP-A mRNA expression levels were found in visceral fat and these were 10-fold higher than in subcutaneous fat. PAPP-A expression significantly increased with age in kidney, brain and gonads. PAPP-A expression significantly deceased with age in bone and skeletal muscle. In the thymus, PAPP-A mRNA showed a biphasic response with age. There were no age-related changes in PAPP-A expression seen in any of the other tissues examined. Expression of IGFBP-5 mRNA, a marker of insulin-like growth factorI (IGF-I) bioactivity known to be regulated by PAPP-A, paralleled the changes in PAPP-A expression with age in kidney, bone, skeletal muscle and thymus. Thus, tissue-specific PAPP-A expression in mice is differentially affected during aging, and may regulate local IGF-I bioactivity in certain tissues.

INTRODUCTION

Pregnancy-associated plasma protein-A (PAPP-A), a novel proteinase in the Metzincin superfamily, is expressed in several human and mouse tissues outside of pregnancy, including those in the cardiovascular, renal, adipose, musculoskeletal and immune systems

Corresponding Author: Cheryl A. Conover, Ph.D., Mayo Clinic, Endocrine Research Unit, 200 First Street SW, 5-194 Joseph, Rochester, MN 55905, Phone: 507-255-6415, Fax: 507-255-4828, Conover.Cheryl@mayo.edu.

Disclosure Statement: The authors have nothing to disclose

(reviewed in 1). In humans, elevated PAPP-A expression has been shown to be associated with acute coronary syndromes and kidney disease (2–8). It was also noted that PAPP-A was highly expressed in human preadipocytes obtained from visceral fat depots compared to those from subcutaneous fat depots (9). Similar findings were reported for mice (10). These studies suggest not only diagnostic and prognostic value, but also potential therapeutic value for PAPP-A. Indeed, in the mouse, global deletion of PAPP-A has been shown to have beneficial effects promoting resistance to atherosclerotic plaque progression, visceral fat accumulation and diabetic nephropathy (8,10,11), and in the maintenance of immune competence with age (12). Furthermore, these PAPP-A knock-out mice live significantly longer than wild-type littermates (13). However, the tissues relevant to these effects are unclear.

Thus, the primary aim of this study was to determine PAPP-A expression in multiple tissues with age in mice, the rationale being that the findings would offer a better understanding of the role of PAPP-A in aging and age-related diseases and provide a scientific basis for targeting strategies that could be translatable to humans.

MATERIALS and METHODS

Mice

Wild-type mice on a mixed C57BL/6, 129 genetic background were used in these experiments. Males and females were housed separately up to five to a cage and fed a standard chow diet. At 1 month, 6 months and 18 months of age, mice were put under deep anesthesia with ketamine/xylazine (90/10 mg/kg) and tissues rapidly excised, snap frozen in liquid nitrogen, and stored at −80°C.

RNA isolation and real-time PCR

Frozen tissues were immediately transferred into 1 ml of Trizol (Life Technologies, Carlsbad, CA) and thoroughly minced. Fat depots, brain, and thymus were homogenized by passing tissue through a 21 gauge needle several times prior to centrifugation. All tissues were centrifuged for 15 minutes at 12,000 rpm. The Trizol layer was extracted into a new tube, 200 μl of chloroform was added to each sample and vigorously shaken for 45 seconds. Samples were allowed to sit at room temperature for 3–5 minutes to allow layers to separate before centrifuging for 15 minutes at 12,000 rpm. The aqueous supernatant was extracted into a clean microcentrifuge tube containing 0.5 ml of isopropanol and vortexed. RNA was allowed to precipitate at room temperature for 10–60 minutes before the RNA was pelleted at 10,000 rpm for 10 minutes. RNA pellets were washed three times with 75% ethanol and allowed to air dry. Approximately 20 μl of molecular grade water was added to each sample. 1 ug of RNA, assessed with a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE), was diluted in 10 μl of molecular grade water and reverse transcribed with the SuperScript® III First-Strand Synthesis System (Life Technologies). PAPP-A mRNA expression was evaluated by quantitative real-time PCR using the iCycler iQ5 Detection System with iQ SYBR green PCR Master Mix (Bio-Rad, Hercules, CA). Amplification plots were analyzed with iQ5 Optical System Software version 2.1 (Bio-Rad). Primer sequences used for mouse PAPP-A, mouse IGFBP-5 and the various reference

genes: TATTA binding protein (TBP), ribosomal protein L22 (RPL22), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and beta-actin (β-ACT) can be found in Table 1. Relative quantification, fold change and statistical significance of gene expression ($P < 0.05$) were determined by the Pfaffl method using the relative expression software tool (REST) 2009 (QIAGEN Inc., Valencia, CA).

RESULTS

PAPP-A expression in tissues of young mice

PAPP-A mRNA expression levels in 1-month-old male and female WT mice are presented in Table 2. The highest PAPP-A expression levels were found in visceral fat, skeletal muscle, bone and testes. High levels were also found in subcutaneous fat, spleen, ovaries, heart, kidney, brain and thymus. PAPP-A mRNA expression was undetectable in liver. There were three tissues that showed significant differences between males and females: gonads, kidney and subcutaneous fat. Males had higher PAPP-A expression (490%) in testes than did females in the ovaries. Females had higher expression of PAPP-A in subcutaneous fat (195%) and kidneys (157%) compared to males.

PAPP-A expression with age

PAPP-A expression significantly increased with age in kidney (~ 2-fold), brain (2- to 4-fold), and gonads (2- to 6-fold) in both males and females (Fig. 1). PAPP-A expression significantly decreased with age in the tibia (70–80%) and quadriceps muscle (~ 40%) in both males and females (Fig. 2). Interestingly, the thymus showed a biphasic effect on PAPP-A mRNA expression with age; expression significantly decreased (~ 50%) between 1 month and 6 months and then increased to the young levels at 18 months in both male and female mice. There were no significant changes in PAPP-A expression across age in adipose tissue, heart, and liver. Thus, PAPP-A mRNA expression remained the highest in visceral fat and undetectable in liver from 1 month to 18 months of age in mice. There was no significant sex*age interaction effect on PAPP-A expression in any of the tissues examined.

IGFBP-5 expression with age

To date, the only established function of PAPP-A is to regulate local IGF bioactivity, i.e., high levels of PAPP-A are associated with increased bioactivity and low levels are associated with decreased bioactivity (1). It is difficult to evaluate IGF-I bioactivity *in vivo*. However, IGFBP-5 is an IGF-I responsive gene, and IGFBP-5 mRNA levels have been used as a surrogate marker for IGF receptor activation in several tissues *in vivo* (11, 14–19). Therefore, we measured IGFBP-5 mRNA in the tissues in Figures 1 and 2 (Table 3). The pattern of IGFBP-5 mRNA across age paralleled that of PAPP-A in kidney, bone, skeletal muscle and thymus. IGFBP-5 expression did not correlate with PAPP-A expression in brain and gonads.

DISCUSSION

In this study we present data on the levels of PAPP-A mRNA expression in multiple tissues of young wild-type mice, both males and females, and differential changes in the expression

with age. Although there have been previous reports assessing PAPP-A mRNA expression in mouse tissues (20–22), these have been limited to semi-quantitative methodologies and/or were assessed at a single young age with insufficient consideration of possible sex-based differences. Thus, the data from this study provide a more comprehensive evaluation of tissue- and sex-specific PAPP-A expression, and for the first time, indicate differential age-dependent changes in PAPP-A expression. The discussion highlights some of the most noteworthy findings.

Sex differences

We saw sex-based differences in PAPP-A expression in three tissues: kidney, subcutaneous fat and gonads. PAPP-A expression was significantly higher in kidneys and subcutaneous fat from females than males. The reason(s) for these differences are unknown. However, sexual dimorphism has been reported for kidney (23), and, in general, females have more subcutaneous fat than males (24). PAPP-A expression was lower in the ovaries compared to the testes, but this may not be an appropriate comparison since these gonadal tissues serve different functions. The rest of the tissues examined had similar levels in both males and females, pointing to a common functionality of PAPP-A.

Tissue differences with age

Visceral fat had the highest expression of PAPP-A mRNA in male and female mice, 4- to 10-fold higher than subcutaneous fat, and the levels did not change significantly with age. Thus, it remained the tissue with the highest level of PAPP-A expression in 18-month-old mice. We have previously shown a preferential impact of PAPP-A gene deletion on visceral fat accumulation in young mice on a high fat diet (10). These PAPP-A knock-out mice also live longer than wild-type littermates when started at 12 months of age on a high fat diet (our unpublished data). Based on these data, one could anticipate targeted PAPP-A inhibition as a feasible approach to limiting visceral obesity and associated morbidity.

The significant up-regulation of PAPP-A expression in the kidney with age was of particular interest. We have previously reported that PAPP-A knock-out mice show decreased incidence and severity of age-related nephropathy (13). Also, 14-month-old female PAPP-A knock-out mice were resistant to experimentally-induced type 2 diabetic nephropathy (8). We had chosen female mice to do the latter studies, since the preliminary data coming out of this study indicated higher levels of PAPP-A expression in females compared to males, and, thus, we reasoned that there might be a greater effect of PAPP-A gene deletion in female mice.

The up-regulation of PAPP-A mRNA expression with age in the brain was also an original finding that warrants further exploration of possible physiological significance. The role of PAPP-A in the brain has not been investigated to date, and it will be important to determine the specific areas of the brain that express PAPP-A, along with the effects of PAPP-A deletion on central regulation of motor function and cognition.

PAPP-A expression in the gonads was high in young mice and somewhat surprisingly increased with age, even though the reproductive capacity is diminished in older mice, especially female mice. We have shown that female PAPP-A knock-out mice have reduced

ovarian function, although they remain fertile (25,26). Male PAPP-A knock-out mice may also have reduced reproductive capacity, although they are able to sire broods. It may be that PAPP-A has an alternative function in these reproductive tissues that could be influenced by age.

On the other hand, PAPP-A mRNA in bone and skeletal muscle significantly decreased with age. Interestingly, PAPP-A expression in heart muscle was not affected by age.

The biphasic change in PAPP-A expression in the thymus, i.e., decreased between 1 and 6 months and increased between 6 and 18 months, was intriguing. The early effects may reflect changes in thymic function during development and the late effects may reflect changes in tissue cellular content, such as adipose tissue infiltration during age-related involution (12). It may be relevant that adipose tissue has high levels of PAPP-A expression as demonstrated in this and a previous study (10).

Liver showed no detectable PAPP-A expression at any of the ages examined. Therefore, liver-specific targeting of PAPP-A is unlikely to have impact, at least in mice.

Mechanism

A major limitation in this study was the lack of a suitable antibody that recognizes mouse PAPP-A, so we were not able to confirm changes in PAPP-A protein at this time. Nonetheless, in this study we used a surrogate marker of PAPP-A regulation of IGF-I bioactivity, IGFBP-5 mRNA levels (11, 14–19). Changes in IGFBP-5 mRNA levels paralleled those of PAPP-A in kidney, bone, skeletal muscle and thymus of both male and female mice, supporting an IGF-dependent role for PAPP-A in these tissues. Previously, Swindell et al. (14) reported a significant decrease in IGFBP-5 mRNA levels in kidneys from 3–6 month old male PAPP-A KO mice compared to wild-type controls. Unlike PAPP-A, there was no change in IGFBP-5 mRNA across age in brain, and IGFBP-5 mRNA levels in the gonads were the exact opposite of PAPP-A suggesting possible IGF-independent effects of PAPP-A.

In conclusion, tissue-specific PAPP-A expression in mice is differentially affected during aging and should be taken into account in future studies designed to target PAPP-A.

Acknowledgments

Funding Source: This work was supported by National Institutes of Health grant AG028141 (to CAC) and the Mayo Foundation. These funding sources had no role in the study design, collection, analysis and interpretation of data, writing of the paper, or decision to submit the article for publication.

References

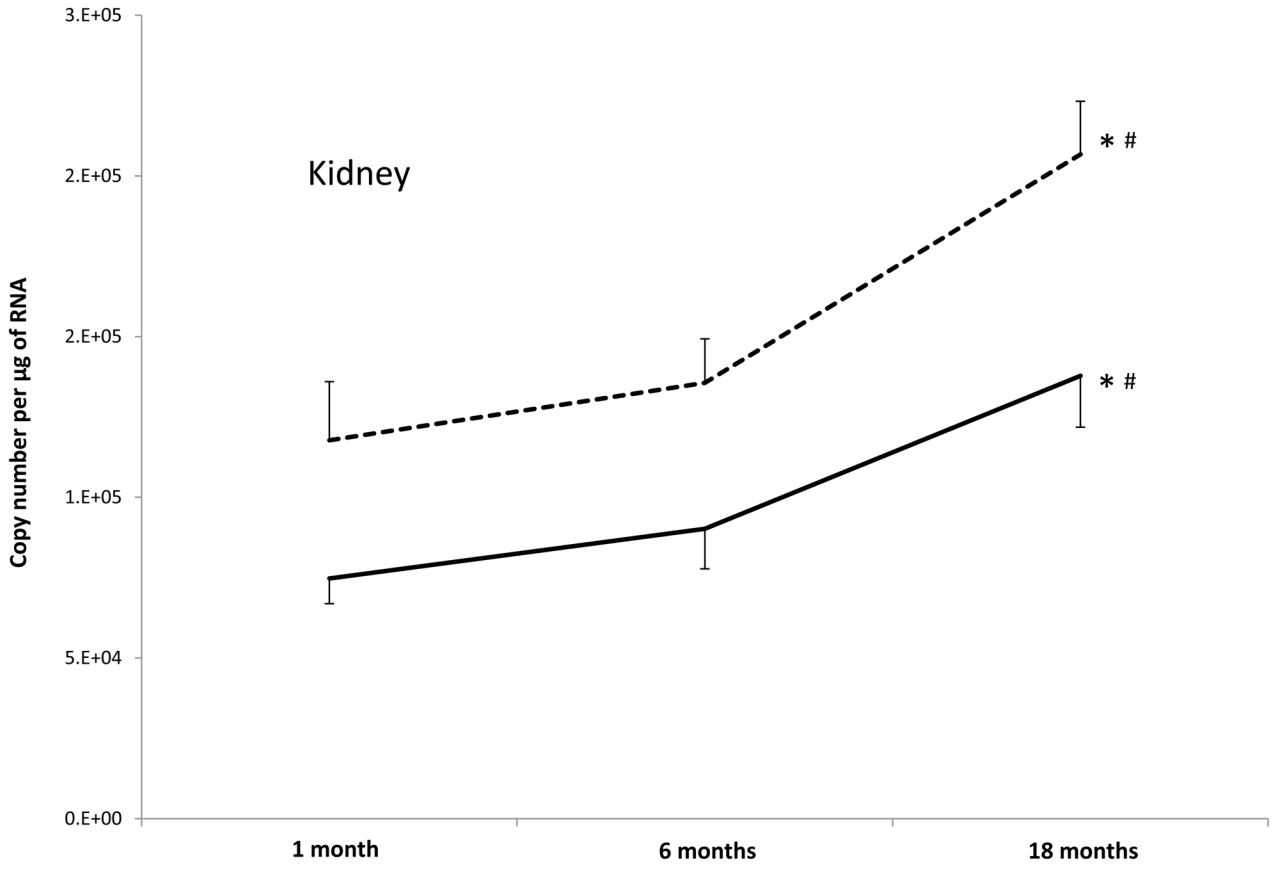
1. Conover CA. Key questions and answers about pregnancy-associated plasma protein-A. Trends Endo Metab. 2012; 23:242–249.
2. Bayes-Genis A, Conover CA, Overgaard MT, Bailey KR, Christiansen M, Holmes DR, Virmani R, Oxvig C, Schwartz RS. Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. N Engl J Med. 2001; 345:1022–1029. [PubMed: 11586954]
3. Consuegra-Sanchez L, Fredericks S, Kaski JC. Pregnancy-associated plasma protein-A (PAPP-A) and cardiovascular risk. Atherosclerosis. 2009; 203:346–352. [PubMed: 18789800]

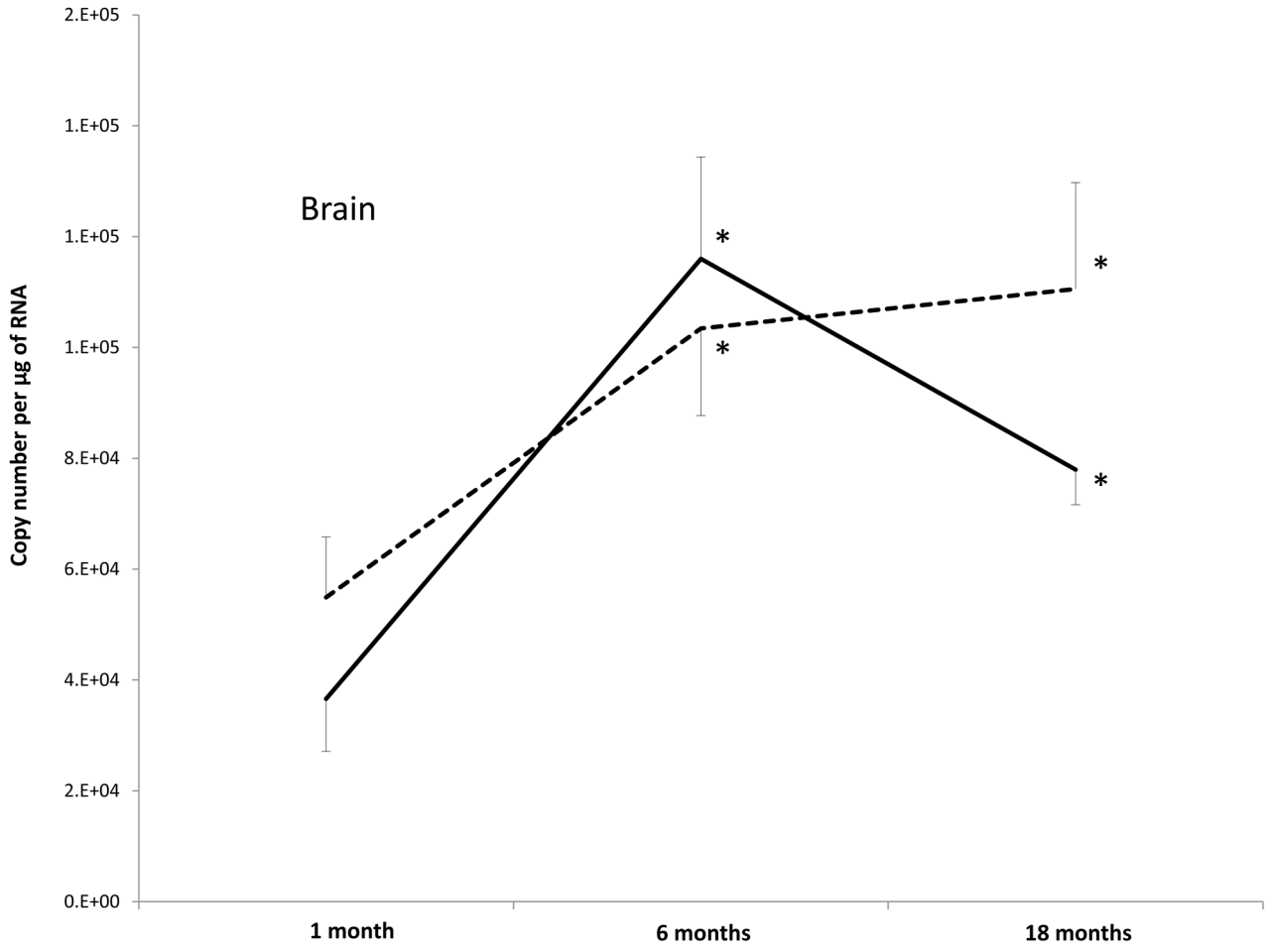
4. Iversen KK, Teisner B, Winkel P, Gluud C, Kjoller E, Kolmos HJ, Hildebrandt PR, Hilden J, Kastrup J. The CLARICOR Trial Group. Pregnancy associated plasma protein-A as a marker for myocardial infarction and death in patients with stable coronary artery disease: a prognostic study within the CLARICOR Trial. Atherosclerosis. 2011; 214:203–208. [PubMed: 21093861]
5. Kalousova M, Vladimir T, Muravka A, Zima T. Pregnancy-associated plasma protein A: spotlight on kidney diseases. Clin Chem Lab Med. 2012; 50:1183–1190. [PubMed: 22850051]
6. Astrup AS, Tarnow L, Christiansen M, Hansen PR, Parving H-H, Rossing P. Pregnancy-associated plasma protein A in a large cohort of type 1 diabetic patients with and without diabetic nephropathy – a prospective follow-up study. Diabetic Medicine. 2007
7. Coskun A, Duran S, Apaydin S, Bulut I, Sariyar M. Pregnancy-associated plasma protein-A: evaluation of a new biomarker in renal transplant patients. Transplantation Proceedings. 2007; 39:3072–3076. [PubMed: 18089325]
8. Mader JR, Resch ZT, McLean GR, et al. Mice deficient in PAPP-A show resistance to the development of diabetic nephropathy. J Endocrinol. 2013; 219:51–58. [PubMed: 23881937]
9. Tchkonia T, Lemburg M, Thomou T, Giorgadze N, Frampton G, Pirtskhalava T, Cartwright M, Flanagan J, Karagiannides I, Gerry N, Forse RA, Tchoukalova T, Pothoulakis C, Kirkland JL. Identification of depot-specific human fat cell progenitors through distinct expression profiles and developmental gene patterns. Am J Physiol Endo Metab. 2007; 209:E298–E307.
10. Conover CA, Harstad SL, Tchkonia T, Kirkland JL. Preferential impact of pregnancy associated plasma protein-A deficiency on visceral fat in mice on high fat diet. Am J Physiol Endo Metabol. 2013; 305:E1145–E1153.
11. Harrington SC, Simari RD, Conover CA. Genetic deletion of pregnancy-associated plasma protein-A is associated with resistance to atherosclerotic lesion development in apolipoprotein E-deficient mice challenged with a high-fat diet. Circ Res. 2007; 100:1696–1702. [PubMed: 17510462]
12. Vallejo AN, Michel JJ, Bale LK, Lemster BH, Borghesi L, Conover CA. Resistance to age-dependent thymic atrophy in long-lived mice that are deficient in pregnancy-associated plasma protein A. Proc Natl Acad Sci USA. 2009; 106:11252–1125. [PubMed: 19549878]
13. Conover CA, Bale LK, Mader JR, Mason MA, Keenan KP, Marler RJ. Longevity and age-related pathology of mice deficient in pregnancy-associated plasma protein-A. J Gerontol: Biol Sci. 2010; 65:590–599.
14. Swindell WR, Masternak MM, Bartke A. In vivo analysis of gene expression in long-lived mice lacking the pregnancy-associated plasma protein A (PappA) gene. Exp Gerontol. 2010; 45:366–374. [PubMed: 20197085]
15. Adamo ML, Ma X, Ackert-Bicknell CL, Donahue LR, Beamer WG, Rosen CJ. Genetic increase in serum insulin-like growth factor-I (IGF-I) in C3H/Hej compared with C57BL/6J mice is associated with increased transcription from the IGF-I exon 2 promoter. Endocrinology. 2006; 147:2944–2955. [PubMed: 16527837]
16. Duan C, Liimatta MB, Bottum OL. Insulin-like growth factor (IGF)I regulates IGF-binding protein-5 gene expression through the phosphatidylinositol 3-kinase, protein kinase B/Akt, and p70 S6 kinase signaling pathway. J Biol Chem. 1999; 274:37147–37153. [PubMed: 10601276]
17. Nichols TC, du Laney T, Zheng B, Bellinger DA, Nichols CA, Engleman W, Clemmons DR. Reduction in atherosclerotic lesion size in pigs by αVβ3 inhibitors in association with of insulin-like growth factor-I-mediated signaling. Circ Res. 1999; 85:1040–1045. [PubMed: 10571535]
18. Resch ZT, Simari RD, Conover CA. Targeted disruption of the pregnancy-associated plasma protein-A gene is associated with diminished smooth muscle cell response to insulin-like growth factor-I and resistance to neointimal hyperplasia after vascular injury. Endocrinology. 2006; 147:5634–5640. [PubMed: 16959843]
19. Ye P, D'Ercole J. Insulin-like growth factor I (IGF-I) regulates IGF binding protein 5 gene expression in the brain. Endocrinology. 1998; 139:65–71. [PubMed: 9421399]
20. Conover CA, Bale LK, Overgaard MT, Johnstone EW, Laursen UH, Fuchtbauer E-M, Oxvig C, van Deursen J. Metalloproteinase pregnancy-associated plasma protein-A is a critical growth regulatory factor during fetal development. Development. 2004; 131:1187–1194. [PubMed: 14973274]

21. Qin X, Sexton C, Byun D, Strong D, Baylink DJ, Mohan S. Differential regulation of pregnancy associated plasma protein (PAPP)-A during pregnancy in human and mouse. GH & IGF Res. 2002; 12:359–366.
22. Conover CA, Boldt HB, Bale LK, Clifton KB, Grell JA, Mader JR, mason EJ, Powell DR. Pregnancy-associated plasma protein-A2 (PAPP-A2): tissue expression and biological consequences of gene knockout in mice. Endocrinology. 2011; 152:2837–2844. [PubMed: 21586553]
23. Bayliss C. Sexual dimorphism in the aging kidney: differences in the nitric oxide system. Nat Rev Nephrol. 2009; 5:384–396. [PubMed: 19488070]
24. Jensen MD. Role of body fat distribution and the metabolic complications of obesity. J Clin Endocrinol Metab. 2008; 93:S57–S63. [PubMed: 18987271]
25. Nyegaard M, Overgaard MT, Su Y-Q, Hamilton AE, Kwintkiewicz J, Hsieh M, Nayak NR, Conti M, Conover CA, Giudice LC. Lack of functional pregnancy-associated plasma protein-A (PAPP-A) compromises mouse ovarian steroidogenesis and female fertility. Biol Reprod. 2010; 82:1129–1138. [PubMed: 20130263]
26. Mason EJ, Grell JA, Wan J, Cohen P, Conover CA. Insulin-like growth factor (IGF)-I and IGF-II contribute differentially to the phenotype of pregnancy associated plasma protein-A knock-out mice. GH & IGF Res. 2011; 21:243–247.
27. Resch ZT, Chen B-K, Bale LK, Oxvig C, Overgaard MT, Conover CA. Pregnancy-associated plasma protein A gene expression as a target of inflammatory cytokines. Endocrinology. 2004; 145:1124–1129. [PubMed: 14657012]
28. Conover CA, Chen B-K, Resch ZT. Regulation of pregnancy-associated plasma protein-A expression in cultured human osteoblasts. Bone. 2003; 34:297–302. [PubMed: 14962808]
29. Conover CA, Bale LK, Harrington SC, Resch ZT, Overgaard MT, Oxvig C. Cytokine stimulation of pregnancy-associated plasma protein A expression in human coronary smooth muscle cells: inhibition by resveratrol. Am J Cell Physiol. 2006; 290:C183–C188.
30. Conover CA, Harrington SC, Bale LK. Differential regulation of pregnancy associated plasma protein-A in human coronary artery endothelial cells and smooth muscle cells. GH & IGF Res. 2008; 18:213–220.
31. Gonzalez C, Auw Yang KG, Schwab JH, Fitzsimmons JS, Reinholtz MM, Resch ZT, Bale LB, Clemens VR, Conover CA, O'Driscoll SW, Reinholz GG. Transforming growth factor-β1 modulates insulin-like growth factor binding protein-4 expression and proteolysis in cultured periosteal explants. GH & IGF Res. 2010; 20:81–86.

***Highlights**

- Tissue-specific expression of PAPP-A indicated variable mRNA levels from highest in visceral fat to undetectable in liver.
- Levels of PAPP-A mRNA expression differed between males and females in kidney, subcutaneous fat and gonads.
- PAPP-A mRNA expression increased with age in kidney, brain, and gonads, and decreased with age in bone and skeletal muscle.
- PAPP-A mRNA expression was biphasic with age in the thymus.
- Change in PAPP-A mRNA across age paralleled that of IGFBP-5 mRNA in kidney, bone, skeletal muscle and thymus.





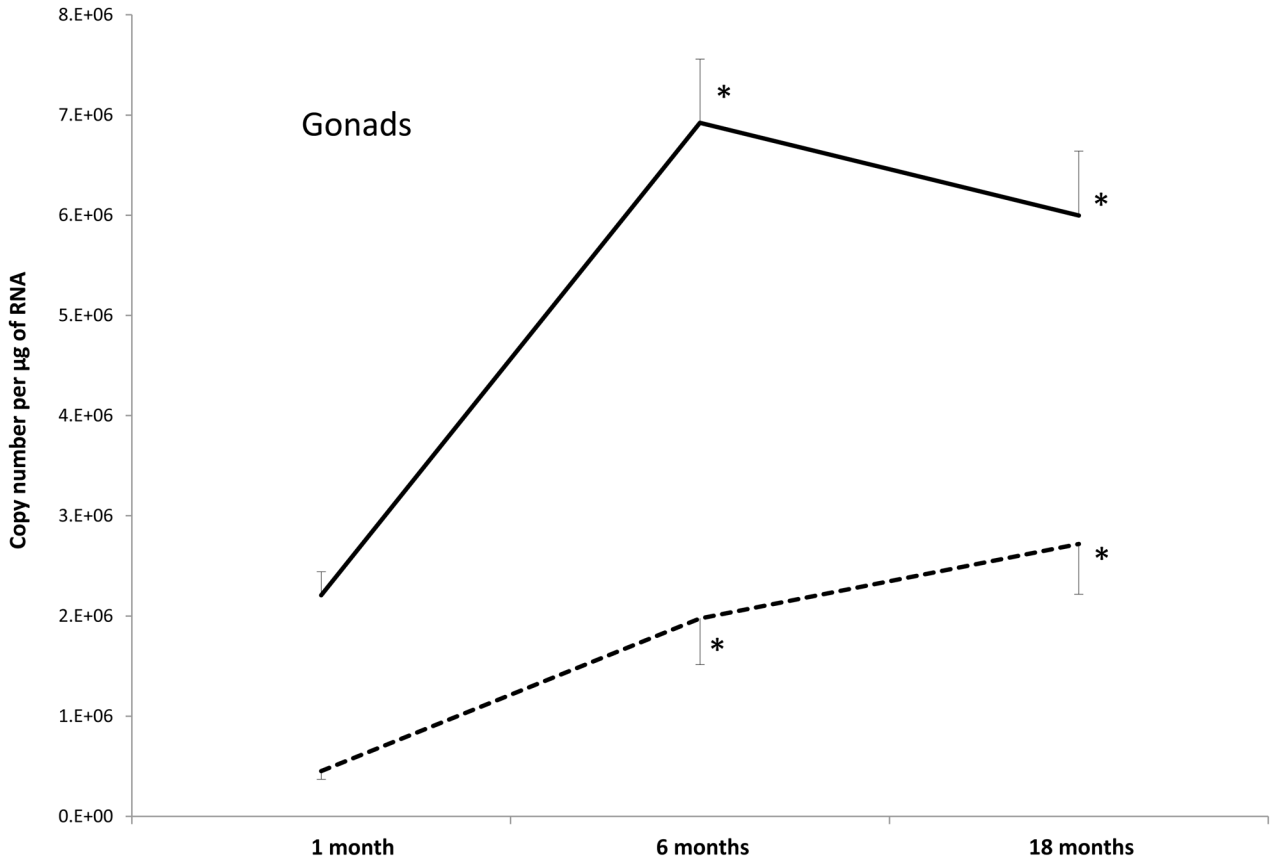


Figure 1.

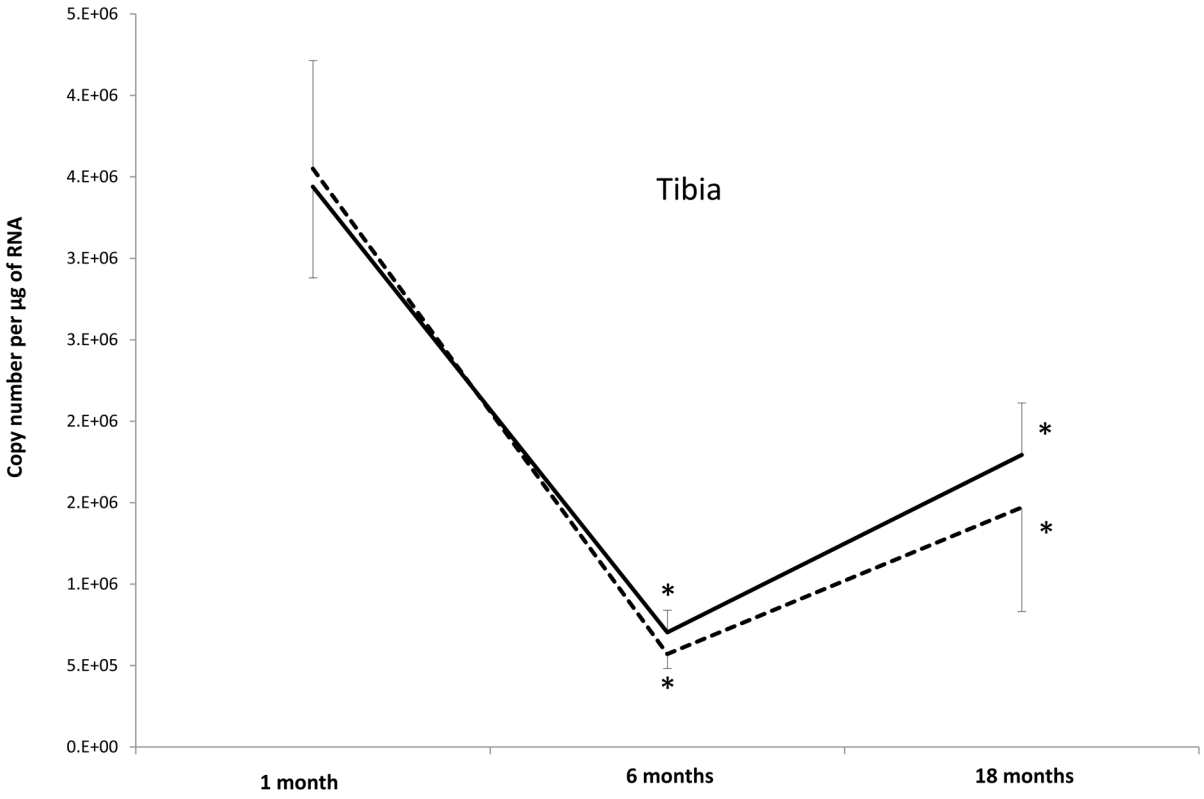
Tissues showing significant increases in PAPP-A mRNA expression with age.

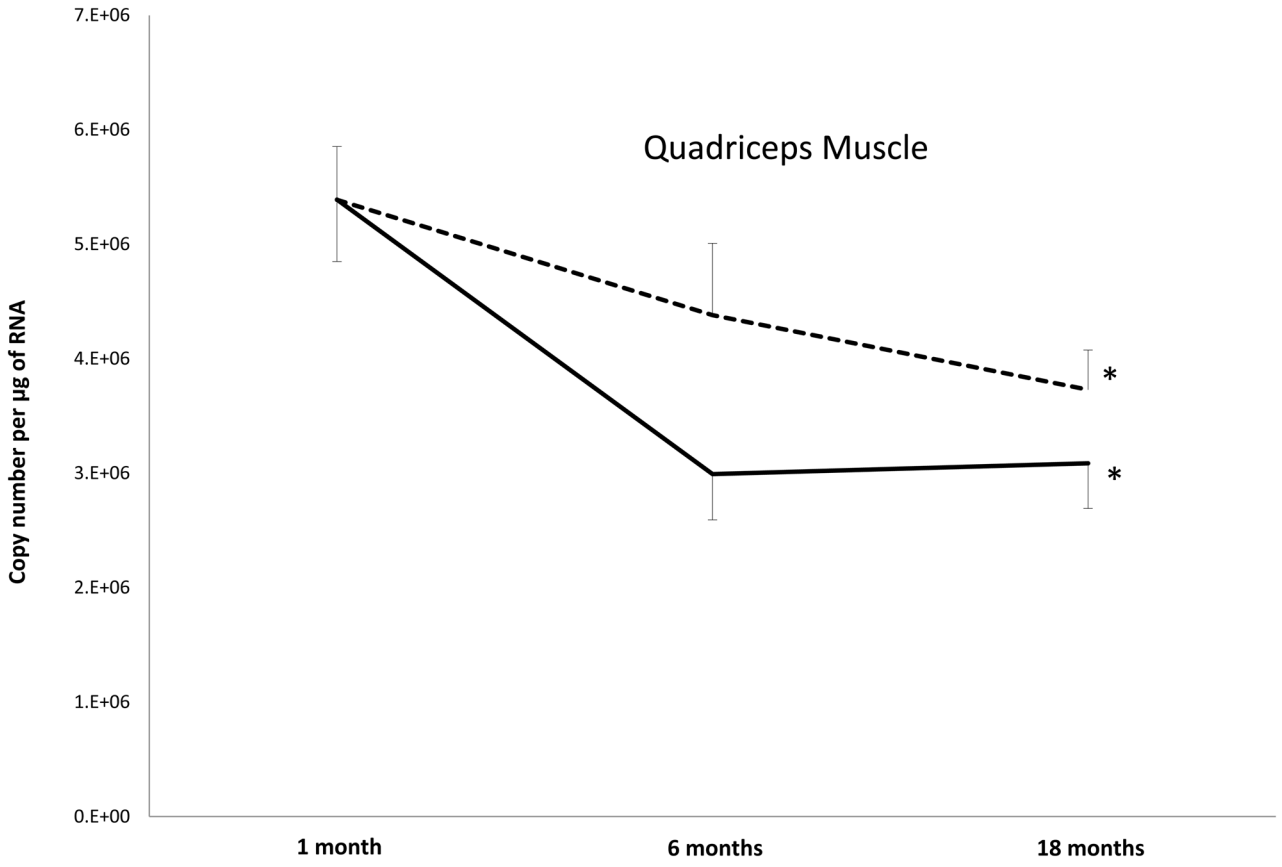
Results are mean ± SEM (N = 5–7).

Males (solid line), Females (dashed line)

*Significantly different relative to 1 month

#Significant difference between 6 months and 18 months





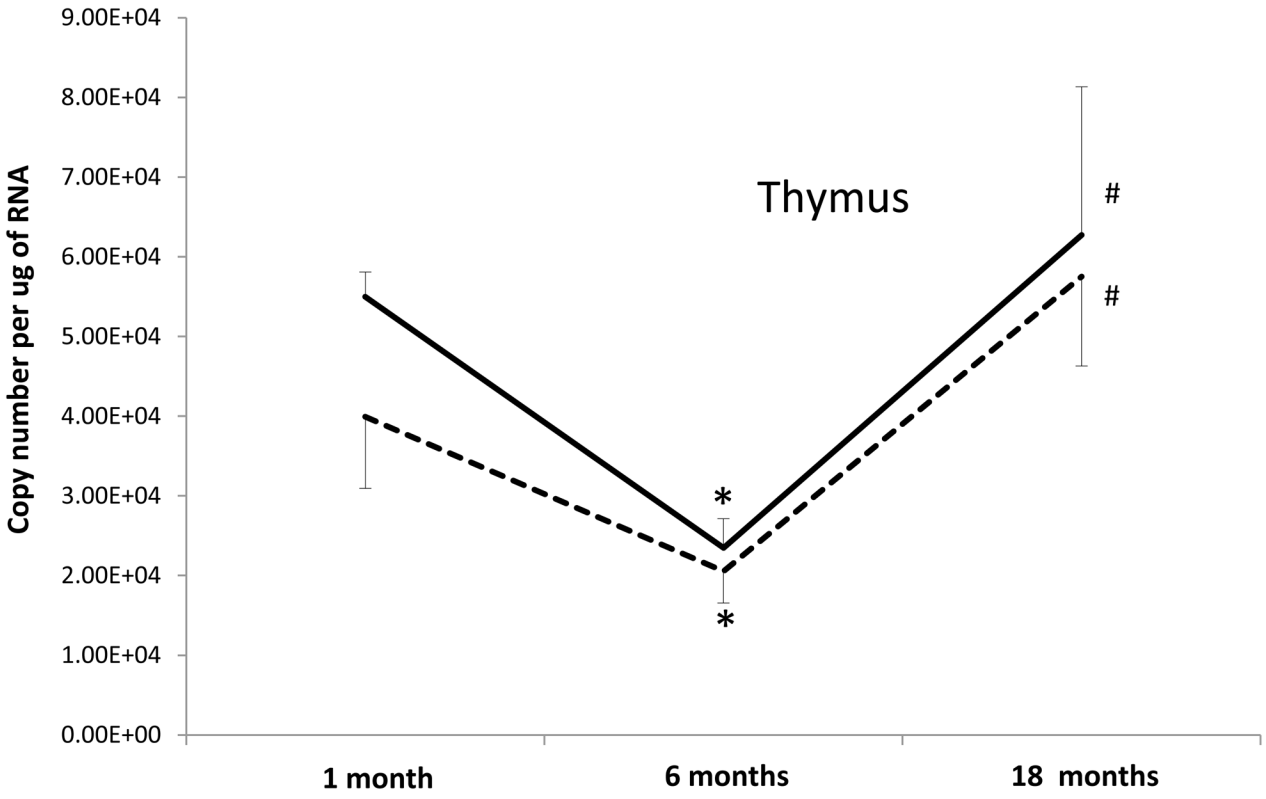


Figure 2.

Tissues showing significant decreases in PAPP-A mRNA expression with age.

Results are mean ± SEM (N = 5–7).

Males (solid line), Females (dashed line)

*Significantly different relative to 1 month

#Significant difference between 6 months and 18 months

Table 1

Real-time PCR Primers

	Gene	NCBI accession number	Forward (5′ to 3′)	Reverse (5′ to 3′)	Size (bp)
Target	PAPP-A	NM_021362.1	gccgtgggagcaatatc	gatggcacactctgaccctat	164
Target	IGFBP-5	NM_010518.2	gaacactgcccaccccagag	ccacgggagggcttacactg	195
Reference	TBP	NM_013684.3	ctcagttacaggtggcagca	cagcacagagcaagcaactc	120
Reference	RPL22	NM_009079.2	ggcggaggagtcgtgacc	ctctcttgctgttggcgaca	158
Reference	GAPDH	NM_008084.2	ttcaccaccatggagaagg	ctcgtggttcacacccatc	111
Reference	B-ACT	NM_007393.3	acaacggctccggcatgtg	tctcttgctctgggcctcgtcac	155

bp, base pairs

Table 2

PAPP-A mRNA expression in tissues of young mice

	Copy number/μg RNA × 10^4	
	Males	**Females**
Visceral Fat	1060 ± 499	712 ± 85
Quadriceps	539 ± 54	539 ± 46
Soleus	373 ± 93	200 ± 39
Tibia	344 ± 56	355 ± 66
Gonads	221 ± 23	45 ± 8*
Subcutaneous Fat	97 ± 20	189 ± 18*
Spleen	38 ± 7	50 ± 6
Heart	34 ± 5	25 ± 3
Kidney	7 ± 0.8	12 ± 2*
Thymus	6 ± 0.3	4 ± 0.9
Brain	4 ± 0.9	5 ± 1
Liver	UD	UD

Results are means ± SEM, N = 5–7

UD, undetected; levels below the lowest standard (5×10^2)

Significant difference between males and females,

*$P < 0.05$

Table 3

IGFBP-5 mRNA expression

Copy number/μg RNA × 10^4						
	Males			**Females**		
	1 month	**6 months**	**18 months**	**1 month**	**6 months**	**18 months**
Kidney	2.4±0.30	5.1±0.60*	7.1±0.31*#	3.2±0.38	5.2±0.62	7.1±1.14*
Brain	3.3±0.47	2.6±0.13	3.7±0.15	3.9±0.22	3.3±0.13	4.1±0.43
Gonads	3.0±0.66	0.4±0.08	0.4±0.15	27.9±2.45	17.1±1.93	18.6±2.71
Tibia	5.2±0.56	4.9±0.31	3.5±0.45*#	6.3±0.65	3.1±0.88*	3.3±0.78
Quadriceps	3.0±0.11	5.5±0.37	3.5±0.79#	4.7±0.92	2.8±0.17	3.0±0.50
Thymus	2.5±0.23	1.4±0.26*	2.8±0.53#	2.5±0.37	1.4±0.30*	4.6±1.29#

Results are means ± SEM N=5–7

*Significantly different from 1 month

#Significant difference between 6 months and 18 months