### P-10-25

# Mitochondrial Apoptosis And Proteolytic Changes Of Myofibrillar Proteins In Two Different Pork Muscles During Aging (#599)

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### Introduction

Apoptosis has been proposed as one of the crucial biochemical processes that possibly involve in the postmortem meat tenderization. During muscle to meat conversion, rapid oxygen and nutrient deprivation could lead to the activation of intrinsic pathway dependent apoptosis, which is closely associated with metabolic status and mitochondrial dysfunction of the muscle cells [1]. Further, early onset of mitochondrial apoptosis and its positive impacts on tenderization has been reported [2]. Given that aging response occurs in a muscle-specific manner, it would be reasonable to postulate that mitochondrial apoptotic changes and their subsequent impacts on proteolysis may be differently affected by different muscle types. Therefore, the objective of this study was to investigate the changes in mitochondrial apoptotic factors, proteolysis and tenderness of two porcine muscles (*Psoas major*; PM and *longissimus dorsi*; LD) during postmortem aging.

#### Methods

Ten pigs (average body weight 115 kg) were harvested, and both PM and LD muscles were collected from the carcasses at various postmortem times (2 h, 6 h, 24 h, 48 h, and 168 h). The samples were analyzed for several biochemical traits, such as mitochondrial membrane permeability, mitochondrial lipid peroxidation, cytochrome c redox stability, Ca<sup>2+</sup> uptake and released, the abundance of cytochrome c, desmin/troponin T degradation, and calpain autolysis. The instrumental tenderness (WBSF) was also determined. Data were analyzed by Two-way ANOVA and PROC MIXED of SAS to compare the traits across muscle types and aging times. Least square means were separated by least significant differences (*P*<0.05).

### Results

Overall, the mitochondrial membrane permeability was increased at 2-168 h postmortem but it became significant at 6-24 postmortem in both two muscle types (*P*<0.05; Table 1). PM had a higher membrane permeability than LD at 24 h postmortem (*P*<0.01). While a significant increase in mitochondrial lipid peroxidation was found in both muscle samples, PM had a greater mitochondrial lipid peroxidation compared with LD at 24 h postmortem (*P*<0.05; Table 1). The cytochrome c redox stability was decreased in both two muscle (*P*<0.05; Table 1). However, LD had a greater cytochrome c redox stability than PM at 6 h (*P*<0.05) and 24 h postmortem (*P*<0.01), respectively. Moreover, while a significant increase in Ca<sup>2+</sup> concentration was found in both muscles, PM had higher Ca<sup>2+</sup> concentration than in LD at 24 h postmore

tem (P<0.05). PM maintained a significantly higher cytochrome c than LD (P<0.01; Fig 1), indicating that the rapid onset of mitochondrial apoptotic process would occur in PM through the release of cytochrome c from mitochondria. While PM had lower WBSF values than LD (P<0.01), PM maintained higher intact desmin and tend to have less degradation of troponin T, less extent of autolyzed products of calpain 1 compared to LD (P<0.01; Table 2). These observations indicate that the rapid onset of mitochondrial apoptosis of PM did not result in an increase in myofibrillar protein degradation of the muscle during postmortem aging.

### Conclusion

The results from the current study found *psoas major* (well-known oxidative muscle) showed the rapid onset of mitochondria apoptotic process compared to *longissimus dorsi* (well-known glycolytic muscle), evidenced by increases in mitochondrial membrane permeability, mitochondrial lipid peroxidation, Ca<sup>2+</sup> concentration and cytochrome c level. However, those increases in apoptotic changes did not result in subsequent increases in myofibrillar protein degradation, indicating perhaps the mitochondrial apoptosis mediated tenderization process could be muscle-specific. Further studies would be warranted to investigate the relationship between muscle-specific mitochondrial apoptosis and energy metabolism and their impacts on meat quality.

#### Acknowledgements

The authors would like to thank the Purdue Meat Laboratory staff and Meat Science and Muscle Biology Lab members at Purdue University for assistance with sample and data collection.

#### References

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# LD PM 2h 6h 24h 168h 2h 6h 24h 168h Reference

# Figure 1. Representative western blots and densitometric analysis of cytochrome c

LD: Longissimus dorsi; PM: Psoas major. Reference was from 2 h postmortem pork PM.

		WBSF (N)	Desmin Intact	Desmin degradation	Troponin T intact	Troponin T degradation	Calpain 80kDa	Calpain 78kDa	Calpain 76kDa
LD	24 h	43.52 <sup>A**</sup>	0.75 <sup>A**</sup>	0.71 <sup>A**</sup>	3.35 <sup>A</sup>	0.42 <sup>B</sup>	0.15 <sup>A*</sup>	0.34 <sup>A**</sup>	0.51 <sup>A**</sup>
	168 h	29.52 <sup>B</sup>	0.61 <sup>A**</sup>	0.71 <sup>A**</sup>	0.53 <sup>B</sup>	2.53 <sup>A**</sup>	0.13 <sup>A*</sup>	0.31 <sup>A**</sup>	0.55 <sup>A**</sup>
РМ	24 h	30.66 <sup>A</sup>	1.44 <sup>A</sup>	0.94 <sup>B</sup>	2.38 <sup>A</sup>	0.27 <sup>B</sup>	0.20 <sup>A</sup>	0.46 <sup>A</sup>	0.34 <sup>B</sup>
	168 h	29.67 <sup>A</sup>	1.09 <sup>B</sup>	1.58 <sup>A</sup>	0.63 <sup>B</sup>	0.46 <sup>A</sup>	0.18 <sup>A</sup>	0.41 <sup>A</sup>	0.42 <sup>A</sup>
P-value	Time	0.0002	0.015	0.087	< 0.0001	0.003	0.202	0.076	0.027
	Muscle	0.001	< 0.0001	0.004	0.270	0.005	0.002	< 0.0001	< 0.0001

A-B Different letters in the same row indicate significant difference between aging time (P<0.05). \*\* indicate significant difference between muscle type (P<0.01), \* indicate significant difference between muscle type (P<0.05).

## Table 2. Changes in instrumental tenderness, proteolysis, and calpain autolysis of muscles

Changes in instrumental tenderness (WBSF), desmin and troponin T degradation, and calpain autolysis of porcine muscles during postmortem aging. Notes

		Aging time (h)							
	Muscle type	2	6	24	48	168			
10.0	LD	0.109±0.007 <sup>A</sup>	0.105±0.005 <sup>A</sup>	0.095±0.005 <sup>B**</sup>	0.086±0.005 <sup>BC*</sup>	0.080±0.004 <sup>C</sup>			
MMP	PM	$0.105 \pm 0.004^{A}$	0.102±0.004 <sup>A</sup>	$0.083 \pm 0.005^{B}$	0.074±0.006 <sup>BC</sup>	0.071±0.005 <sup>C</sup>			
MLP (mg MDA/kg	LD	1.408±0.091 <sup>C</sup>	1.441±0.073 <sup>C</sup>	1.689±0.083 <sup>B*</sup>	1.889±0.086 <sup>A</sup>	1.796±0.082 <sup>AB</sup>			
meat)	PM	1.432±0.082 <sup>B</sup>	1.524±0.071 <sup>B</sup>	1.872±0.082 <sup>A</sup>	$1.815 \pm 0.087^{A}$	1.776±0.103 <sup>A</sup>			
CRS (Δ550-535/mg	LD	0.444±0.038 <sup>A</sup>	0.395±0.056 <sup>A*</sup>	0.278±0.052 <sup>B**</sup>	0.250±0.042 <sup>B</sup>	0.285±0.108 <sup>AB</sup>			
protein)	PM	0.391±0.032 <sup>A</sup>	$0.289 \pm 0.038^{B}$	0.169±0.035 <sup>C</sup>	0.153±0.048 <sup>C</sup>	0.134±0.049 <sup>C</sup>			
C-3+ ITD	LD	0.065±0.002 <sup>C</sup>	0.067±0.004 <sup>C</sup>	0.075±0.003 <sup>B*</sup>	0.085±0.003 <sup>AB</sup>	0.087±0.005 <sup>A</sup>			
Ca <sup>2</sup> UK	PM	0.067±0.004 <sup>C</sup>	0.074±0.003 <sup>C</sup>	0.083±0.003 <sup>AB</sup>	$0.087 \pm 0.004^{A}$	0.092±0.003 <sup>A</sup>			
C. 4 1	LD	0.264±0.124 <sup>A*</sup>	0.392±0.184 <sup>A**</sup>	0.438±0.283 <sup>A**</sup>	NA	0.300±0.192 <sup>A*</sup>			
Cytochrome c	PM	0.639±0.343 <sup>B</sup>	1.126±0.443 <sup>A</sup>	1.204±0.718 <sup>A</sup>	NA	$0.617 \pm 0.094^{B}$			

A-C Different letters in the same row indicate significant difference between aging time (P<0.05). \*\* indicate significant difference between muscle type (P<0.01), \* indicate significant difference between muscle type (P<0.05).

# Table 1. Changes in MMP, MLP, CRS, and Ca2+ UR of porcine muscles during postmortem aging.

LD: Longissimus dorsi; PM: Psoas major; MMP: Mitochondrial membrane permeability; MLP: Mitochondrial lipid peroxidation; CRS: Cytochrome c redox state; Ca<sup>2+</sup> UR: Ca<sup>2+</sup> uptake and release. Notes