



REVIEW

The Role of Meat Protein in Generation of Oxidative Stress and Pathophysiology of Metabolic Syndromes

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Abstract Various processing methods have a great impact on the physiochemical and nutritional properties of meat that are of health concern. Hence, the postmortem processing of meat by different methods is likely to intensify the potential effects on protein oxidation. The influence of meat protein oxidation on the modulation of the systemic redox status and underlying mechanism is well known. However, the effects of processed meat proteins isolated from different sources on gut microbiota, oxidative stress biomarkers, and metabolomic markers associated with metabolic syndromes are of growing interest. The application of advanced methodological approaches based on OMICS, and mass spectrometric technologies has enabled to better understand the molecular basis of the effect of processed meat oxidation on human health and the aging process. Animal studies indicate the involvement of dietary proteins isolated from different sources on health disorders, which emphasizes the impact of processed meat protein on the richness of bacterial taxa such as (*Mucispirillum*, *Oscillibacter*), accompanied by increased expression of lipogenic genes. This review explores the most recent evidences on meat processing techniques, meat protein oxidation, underlying mechanisms, and their potential effects on nutritional value, gut microbiota composition and possible implications on human health.

Keywords diet, processed meat protein, oxidative stress, metabolic syndromes

Introduction

Meat and meat products do not only contain valuable nutrients for human health including vitamins (niacin, thiamin, riboflavin, vitamin B₆ and vitamin B₁₂), but also contain sodium, heme iron, advanced glycation end products, cholesterol and saturated fatty acids that may be harmful for patients with non-alcoholic fatty liver disease

(NAFLD) (Miele et al., 2014; Oddy et al., 2013). Excess consumption of red meat is associated with type 2 diabetes (T2D), insulin resistance (IR) and NAFLD (Hernandez et al., 2017; Zelber-Sagi et al., 2018). Meat products go through many processing steps either industrially or in households, most of which may trigger protein oxidation (Estevez, 2011). Physiochemical and functional changes such as aggregation, fragmentation and modification of structure during processing of meat may cause protein oxidation (Kaur et al., 2014; Sayd et al., 2016). Aging and age-related diseases have been associated with the biological consequences of such chemical changes (Shacter, 2000; Stadtman, 2006). Recent reports have hypothesized whether the occurrence of protein oxidation may not only affect sensory and technological attributes of the meat but also the health and well-being of the consumers (Estevez, 2011). Although more challenging and novel advances have recently been accomplished by food scientists in this field to better understand the effects of protein oxidation on the biomarkers associated with human health. Succeeding studies have been conducted to understand the various effects of protein oxidation on food quality, including altered texture and impaired digestibility (Sante-Lhoutellier et al., 2007).

Oxidized proteins are involved in the onset and severity of different diseases. And it is reasonable to hypothesize that the intake of oxidized proteins may be associated with certain pathological conditions and *in vivo* oxidative stress. This is supported by the growing concern about the impact of animal source proteins on human health, given that such proteins are particularly susceptible to oxidation (Estevez, 2015; Estrada et al., 2018). Studies have reported that aging related oxidation of protein is reduced by dietary protein restriction, which clearly emphasizes the link between protein intake and *in vivo* protein oxidation (Youngman et al., 1992). Owing to high levels of protein carbonyls, processed meat proteins are probably the most remarkable sources of the dietary protein oxidation (Jiang and Xiong, 2016; Soladoye et al., 2015). Higher intake of red meat has been found to increase inflammatory markers and systemic oxidative damage to lipids and proteins in experimental animals (Jakobsen et al., 2017; Turner et al., 2017). Recently, a study has shown that proteins isolated from meat sources have different impacts from soy and casein proteins on muscle anti-oxidation, liver metabolism and gut microbiota composition (Zhu et al., 2017). On the contrary, intake of soy protein has negligible effects on blood lipids and oxidative stress biomarkers (Engelman et al., 2005). It is worth recalling that epidemiological studies and reports from health authorities claim on the connection between red and processed meat with obesity and other serious pathologies (Chan et al., 2011; IARC, 2015). Recent studies have revealed that processed meat proteins isolated from different sources exhibited different impact on the intestinal gut microbiota and NAFLD (Ahmad et al., 2019; Ijaz et al., 2018). The bacterial taxa such as *Tenericutes*, *Christensenellaceae*, and *Akkermansia* have been reported to be associated with lipid metabolism and high-density lipoproteins (HDL) (Everard et al., 2013; Fu et al., 2015). Also, *Akkermansia muciniphila* can reduce the level of liver triglyceride and gonadal fat mass (Org et al., 2015). In a recent study, carnitine is an abundant nutrient in red meat, produces trimethylamine-N-oxide via gut microbiota metabolism of L-carnitine, which has been shown to promote cardiovascular disease risks in host (Koeth et al., 2013). However, the effects of dietary proteins isolated from different sources on oxidative stress biomarkers, gut microbiota composition, NAFLD, and metabolic risk markers remains to be established.

Hence, this paper concisely collects recent advances on the effects of dietary processed meat proteins isolated from different sources on the host microbial balance, gut-liver axis and NAFLD. In addition, we have also discussed the underlying mechanisms on the associations between the protein oxidation and oxidative stress. We suggest reasonable hypotheses and future challenges intended to stimulate further investigation in this emerging field of research.

Mechanism Underlying the Protein Oxidation

Proteins are recognized as major targets of oxidative modification owing to a variety of mechanisms (Dean et al., 1997). It

has been well known that oxidation products and oxidation pathways are dependent on how and where the oxidation is initiated on proteins (Xiong et al., 2009; Xiong et al., 2000). Both physical (UV light and fluorescent) and chemical (reducing sugars, Millard reaction, dicarbonyls from lipid oxidation, and ROS) agents trigger protein oxidation in food system (Estevez et al., 2015; Soladoye et al., 2015). In theory, peptide scission, covalent interactions between amino acids chains, and formation of crosslinks collectively lead to the formation of specific oxidation derivatives (Lund et al., 2011). In practice, protein carbonylation is a well-used marker for oxidative damage to food proteins, together with the crosslinks formation, namely, dityrosines and disulphide bonds (Estevez et al., 2015). Furthermore, carbonylation has been observed in assorted muscle foods such as beef patties, fermented sausages, bacon, and milk and dairy proteins (Berardo et al., 2016; Rysman et al., 2016; Soladoye et al., 2017; Utrera et al., 2015). More sensitive and high throughput mass spectrometric technology has been applied to identify and locate protein carbonylation in dairy and pork products (Milkovska-Stamenova et al., 2017; Mitra et al., 2018). In fresh meat, protein carbonylation has been widely accepted as oxidative stress biomarkers, and can vary with species, muscle type and aging time. For example, carbonyl content in meat myofibrils varies from 0.2 nmol/mg to 4.8 nmol/mg protein in lamb *longissimus dorsi* muscle and bovine diaphragm pedialis muscle respectively (Martinaud et al., 1997; Sante-Lhoutellier et al., 2008).

Postmortem meat processing is usually done to alter or enhance the nutritional composition of meat. The loss of sulfhydryl (SH) group is detected as another marker of oxidative modification of proteins. Methionine loss in meat systems, which is very sensitive amino acid to ROS, is a major cause of oxidation of meat proteins. The observed consequences during cooking are loss of SH groups and increased surface hydrophobicity (Gatellier et al., 2010). In addition to cooking conditions, studies have shown that a high ionic strength environment increased oxidation of muscle proteins (Liu et al., 2011). Also, cooked pork showed significantly higher β -sheet, β -turn and random coil contents, and lower α -helix content than emulsion type sausage (He et al., 2018). In that study, Raman spectroscopy was used to determine the protein conformation, and moreover, the digestion products were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results showed that stewed pork, cooked pork, and emulsion-type sausage pork had significantly lower levels of SH contents compared to raw pork, but did not show significant differences in dry-cured pork. Several factors may cause differences in the SH contents. The structure of protein is affected by cooking temperature. Heating meat protein at 70°C causes oligomers formation and protein unfolding, while at 100°C or higher, the proteins are further modified by oxidation, which promotes aggregation (Liu et al., 2011). Protein aggregation is a long-lasting problem induced by cooking time and temperature (Philo et al., 2009). In a study, dry-cured pork had different SH contents compared to cooked pork, although both types of proteins were heated under the same conditions. Similarly, the SH contents of dry-cured pork were significantly higher than those of other pork products. This mechanism might be attributed to a looser or more disrupted myofibril structure caused by electrostatic repulsion in the high-ionic strength salty environment (Bax et al 2012; Philo et al., 2009). These results indicated that during cooking moderate unfolding and denaturation occurred in meat protein.

Protein digestibility and particle size of pork products is affected by method of processing (Soladoye et al., 2015). Also, methods of cooking affect *in vitro* protein digestibility and availability (Li et al., 2017). Chopping and drying affect the digestibility of protein (Wen et al., 2015). The processing methods not only affect the protein digestibility of meat products but also the peptide fractions released after digestion, and these changes in protein structure and surface hydrophobicity can be attributed to protein oxidation and aggregation (Berardo et al., 2017; Sun et al., 2011). In a study, emulsion-type sausage showed significantly higher protein digestibility, while the lowest protein digestibility was obtained after pepsin digestion alone or with subsequent trypsin digestion or under both these conditions (Li et al., 2017). However, it is not clear how

protein digestibility was affected by different cooking conditions such as time and temperature. In a study, the authors compared the effects of four kinds of processed pork products (dry-cured pork, cooked pork, stewed pork and emulsion-type sausage) on the protein digestibility and digested products (Li et al., 2017). The lowest protein digestibility and the highest particle size were observed in stewed pork, while the opposite for emulsion-type sausage. These results indicated that *in vitro* protein digestibility of pork products can be affected by different processing methods.

The mechanism of lipid peroxidation is a chain reaction and created by a free radical chain reaction (Lund et al., 2011). In a complex matrix such as meat, the associations between protein oxidation and lipid peroxidation are still unclear. The process of protein oxidation leads to the onset of lipid oxidation (Kaur et al., 2014). Moreover, lipid peroxidation products are more susceptible to reacting with amino acids having reactive side chains. Though lipid peroxides are detected earlier than those from the protein oxidation, the oxidation of lipids, specifically hydroxyl radical would react faster with certain amino acid residues (thiols, tryptophan) than with unsaturated fatty acids (Aalhus et al., 2014).

The importance of heme iron in the co-oxidation of lipids and proteins in meat system using pork homogenate has been reviewed (Jongberg et al., 2011; Skibsted, 2011). Fe^{2+} promotes the oxidation of lipids and proteins in a dose-dependent manner and highlights the pro-oxidant role of oxymyoglobin in such reactions. The functionality and digestibility of food proteins such as soy, meat, fish, milk, and myoglobin were shown to be affected by formation of 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA) via covalent modification (Gurbuz and Heinonen, 2015; Hu et al., 2017; Suman et al., 2014; Zhang et al., 2017; Zhou et al., 2015).

Protein Oxidation and Metabolic Disorders

Animal-based protein is an important dietary source for human nutritional requirements. Meat protein distinguishes itself for its richness in all the essential amino acids (Smith et al., 2009). However, except for their differences in amino acid composition, the impact of dietary proteins isolated from different sources on metabolic disorders needs further investigation. Recently, Song et al. (2016) compared the effects of soy and meat proteins on hepatic transcriptomic and metabolic syndrome associated with physiological markers. Functional classification revealed that soy and meat proteins differentially regulate pathways involving amino acid metabolism, energy metabolism, lipid metabolism and insulin signaling. Rictor, Srebf1, NFE2L2, and ATF4 were recognized as potential key upstream regulators. In another study, Song et al. (2018) observed that meat proteins showed beneficial impacts on growth and metabolism in young rats compared to soy and casein proteins.

The pathophysiology and progression of NAFLD is influenced by multiple factors in which oxidative stress plays a key role in the hepatic injury (Oliveira et al., 2002; Wruck and Adjaye, 2017). Another factor associated with hepatic inflammation and lipotoxicity is the stimulation of ROS in fatty liver via mitochondrial dysfunction (Roskams et al., 2003). Intracellular ROS generation and redox homeostasis is regulated by the balance between antioxidant enzymes and ROS generating enzymes, including catalase, glutathione peroxidases (GPX), glutathione (GSH), superoxide dismutase (SOD), and oxidoreductase protein families: glutaredoxin (Glx) and thioredoxin (Mann et al., 2017). Glrx is a multi-effect cytokine that takes part in cell signaling, protection of cell against oxidative stress, cytoskeletal regulation, and inflammation (Aesif et al., 2011; Shelton et al., 2007). Quantitative metabolomics and proteomics analyses highlighted that cellular depletion of Glrx1 activates p53 and associated signaling pathways but decreased the level of GSH (Yang et al., 2018). Glrx1 is also a potential biomarker and key factor involved in the pathogenesis of diabetes and chronic kidney disease (Du et al., 2013). In a study, Glrx1 knockdown increased the levels of GPX, SOD and catalase, but decreased the levels of ROS and MDA compared with

the wild-type mice (Ahmad et al., 2019). A previous study shows that depletion of *Glrx1* results in higher level of ROS and lower level of antioxidant enzymes (Catalase, SOD, and GPX) activities in mice (Liu et al., 2016). This might be because liver contains abundant *Glrx* and more significantly, has two reactive cysteines (Ley et al., 2016) and explain why mice shows increased hepatic antioxidant defense.

Dietary Protein and Gut-liver Axis

The gut is a complex ecosystem that harbors a diverse bacterial community. Recent studies have revealed that *Bacteroidetes* and *Firmicutes* phyla are the most abundant in gut (Moschen et al., 2013). Significant progress has been made in recent years in elucidating the association of gut microbiota composition with severity of NAFLD (Backhed, 2004; Tremaroli et al., 2012). Alterations of gut microbiota have been thought to be associated with a decrease in the ratio of *Bacteroidetes* to *Firmicutes*, gut bacterial richness and increased expression of genes related to bacterial metabolic activity (Ley, 2006; Le Roy, 2013). In a study, male rats fed casein and soy protein diets showed differences in the biochemical markers associated with hepatic antioxidant enzyme activities and metabolism, accompanied with an increase in the abundances of several taxa such as *Ruminococcus* and *Lactobacillus* (Zhu et al., 2017). However, associations among dietary intakes of processed meat proteins as a result of protein oxidation, host health and gut microbiota composition are still unclear. Our studies highlighted that intake of processed meat protein significantly increased the richness of bacterial taxa (*Mucispirillum*, *Oscillibacter*) associated with obesity and NAFLD, which were also accompanied by an increase in the lipogenic gene expression (Ahmad et al., 2019; Ijaz et al., 2018). Still, the effects of dietary proteins isolated from different sources at different levels incorporated with low or high fat is a matter of further discussion.

Studies showed positive associations among processed meat proteins, oxidative stress, and NAFLD (Kirpich et al., 2015). Excess consumption of processed and red meat increases the risk of a serious liver condition and IR. A study revealed that people who ate the maximum amount of red and processed meats had almost a 50% increased risk to NAFLD, and a higher risk to progressing IR (Zelber-sagi et al., 2018). It was also observed that meat cooked at high temperature for a long duration such as grilling, broiling, or frying was related to about twice the risk of IR. Consequently, levels of serum and hepatic inflammatory mediators (TNF- α , MCP-1) have been found to elevate with both steatosis and NAFLD (Ijaz et al., 2018). More recently, consumption of simple carbohydrate has been pointed out as another possible contributory factor. Multiple diet-induced animal models have been developed for the study of NAFLD. Our previous study demonstrated that high fat beef protein diets increased hepatic triglycerides, total cholesterol, LDL-cholesterol, serum inflammatory markers, hepatic lipid accumulation and upregulated lipogenesis genes (Ahmad et al., 2019). As a result, mice fed high fat beef protein exhibited signs of impaired glucose metabolism and IR compared to high fat diets incorporated with soy and casein protein.

Conclusion

There is a growing interest in processed meat proteins and impact of processing methods on nutrition and health. A full understanding of the chemistry behind the meat protein oxidation is paramount in terms of food quality and consumer health. Oxidative and nitrosative modification of proteins in muscle is a topic to be further explored. The identification of chemistry fundamentals of protein oxidation products in processed meat is required to assess their potential toxicity. Although the effects of isolated dietary protein from different sources by our group open a new chapter to better understand the underlying mechanism and potential pathways on gut health and different axis in the field of nutrigenomics. Future studies are needed to

better understand the effects of processing methods on the dietary protein composition, nutritional value and their consequences. Furthermore, meta-proteomic, metabolomic, and meta-transcriptomic studies are needed to identify the changes in the gut microbiota as a result of dietary challenge.

Conflict of Interest

The authors declare no potential conflict of interest.

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Author Contributions

Conceptualization: Li C, Ahmad MI. Data curation: Ijaz MU. Software: Ahmad MI, Li C. Validation: Li C. Investigation: Ahmad MI, Ijaz H. Writing-original draft: Ahmad MI, Li C. Writing-review & editing: Ahmad MI, Ijaz MU, Ijaz H, Li C.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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