Review

Novel aspects of health promoting compounds in meat


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Abstract

Meat is an integral part of the human diet. Besides essential amino acids and nutritive factors of high quality and availability, meat provides often overlooked components of importance for human health. These are amino acids and bioactive compounds that may be very important in i) preventing muscle wasting diseases, such as in sarcopenia, ii) reducing food and caloric intake to prevent metabolic syndrome, iii) blood pressure homeostasis via ACE-inhibitory components from connective tissue, and iv) maintaining functional gut environment through meat-derived nucleotides and nucleosides. In addition, meat could be an important source of phytanic acid, conjugated linoleic acids and antioxidants. Further, it becomes increasingly apparent that design of in vitro meat will be possible, and that this development may lead to improved health benefits from commercially viable and sustainable meat products.

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Contents

1. Introduction .............................................................. 904
2. Amino acids and peptides in sarcopenia ................................................. 905
3. Protein hydrolysates .......................................................... 905
   3.1. Physiologic activities of meat protein hydrolysates in GI ...................................... 906
   3.1.1. Satiety ......................................................... 906
   3.1.2. Amino acid and peptide sensing in GI is mediated by taste receptors .................... 906
4. ACE-inhibitory components from connective tissue ............................................ 906
5. Nucleotides and nucleosides ....................................................... 906
6. Phytanic acid .............................................................. 907
7. Conjugated linoleic acids ........................................................ 907
8. Antioxidants .............................................................. 908
9. In vitro meat production and nutritional design .............................................. 908
   9.1. Production ............................................................ 908
   9.2. From cell culture to meat ..................................................... 908
   9.2.1. Choice of cell type .................................................... 908
   9.2.2. Scaffold ......................................................... 908
   9.2.3. Maturation ....................................................... 908
   9.2.4. Nutritional value .................................................... 908
10. Conclusion ............................................................... 909
References .................................................................. 909

1. Introduction

The unique status of meat in the diet is indisputable as it is the commodity providing the highest amount of protein per unit energy as well as the best quality of protein including all essential amino acids in adequate proportions (Schonfeldt & Hall, 2012). However, general overconsumption of meat, preparation at high temperatures, processing such as addition of salt and nitrate as well as its high content of saturated fat has contributed to the general perception among consumers in the developed part of the world that meat consumption should be reduced for health reasons. This perception emanates from
scientific hypotheses supported by numerous epidemiological cohort studies, but methods as well as inclusion/exclusion criteria of these types of studies are often debated (McNeill & Van Elswyk, 2012). A recent meta-analysis, where fresh meat was analyzed separately from processed meat, revealed little or no increased risk of coronary heart disease (CHD) or type II diabetes associated with unprocessed meat consumption, whereas a higher risk of developing CHD was associated with processed meat, presumably due to the relatively high content of sodium (Micha, Michas, & Mozaffarian, 2012).

Some of the most intensively investigated issues in relation to meat consumption and health aspects are means of reducing formation of heterocyclic aromatic amines (Dundar, Saricoban, & Yilmaz, 2012; Viegas, Amaro, Ferreira, & Pinho, 2012), possible manipulation of lipid composition (Mapiye et al., 2012; Pestana et al., 2012) and reduction of salt content (Ferrini, Comaposada, Arnau, & Gou, 2012; Guardia, Guerrero, Gelabert, Gou, & Arnau, 2006).

Hence, much focus in meat and health research has been on damage control instead of focusing on possible nutritionally advantageous aspects of meat consumption. This is much in contrast to the impressive amount of scientific evidence being collected on the positive aspects of fruit and vegetable consumption on human health. However, a growing interest for innovation in animal-derived products is emerging and it would be profitable to focus our efforts on some of the health promoting aspects of meat consumption, some of which have so far received scant attention. We have chosen to briefly highlight some aspects of meat research that we find promising and worthy of further investigation.

Meat can be seen as an important source of 1) amino acids and peptides in sarcopenia, 2) protein for bioactive hydrolysates, 3) ACE-inhibitory components from connective tissue, as well as 4) nucleotides and nucleosides for gut health. In addition, meat may be an important source of 5) phytanic acid, 6) conjugated linoleic acids and 7) antioxidants. Further, we will probably in the future be able to 8) design in vitro meat to improve the health beneficial aspects of meat. These issues will be presented in the present review.

2. Amino acids and peptides in sarcopenia

Sarcopenia is an age-related muscle-wasting syndrome characterized as a reduction in muscle mass and strength with adverse outcomes such as physical disability, poor quality of life and morbidity (Cruz-Jentoft et al., 2010). Sarcopenia may be exacerbated by simultaneous obesity (sarcopenic obesity) (Thornell, 2011) and sarcopenia may in turn aggravate diabetes (Srikanthan, Hevener, & Karlamangla, 2011). Also chronic illness, e.g. cancer and HIV, may result in lower muscle mass and strength known as cachexia (Sakuma & Yamaguchi, 2012). In case of sarcopenia, muscle mass decreases about 0.5–1.0% per year beginning at the age of 40 (Paddon-Jones, Short, Campbell, Volpi, & Wolfe, 2008). The underlying mechanism(s) are unknown but it has been speculated that age-related changes in food intake, physical exercise, loss of muscle fibers, chronic inflammation, and hormones may be contributing factors. Treatment with testosterone and growth hormone only moderately affect muscle mass and strength in elderly while treatment with growth hormone releasing hormone (GHRH) is promising (Borst, 2004). However, a focused dietary intervention may have the potential to counteract the onset or alleviate sarcopenia, at least to some extent.

Recommended daily intake (RDI) for protein is 0.8 g/kg/day, but studies suggest that a slight increase of this recommendation to 1.0–1.3 g/kg/day could reduce muscle loss with age (Paddon-Jones et al., 2008). Not only is quantity important for the RDI, but also the quality as reflected in digestibility and amino acid composition. Meat is an excellent protein source in this respect, and also contains large amounts of the functional amino acid leucine, which stimulates protein synthesis by mTOR signaling (Du, Shen, Zhu, & Ford, 2007).

Thus, it was found that a moderate intake of lean beef could increase muscle protein synthesis in both young and elderly men and women (Paddon-Jones et al., 2008). The most efficient intervention towards sarcopenia is resistance training. However, although not consistent, results have been published showing a synergistic effect of increased meat intake and increased muscle mass in older men (Evans, 2004).

Overall sarcopenia is due to an imbalance in protein turnover. The satellite cell is important for the turnover of proteins in muscle tissue, however it is not likely that the satellite cell is involved in the development of sarcopenia but rather that activation of satellite cells is indispensable for the muscle anabolism following resistance training (Thornell, 2011). Interestingly meat hydrolysates contain ACE inhibitors (Sections 2 and 3), and it was recently found that treatment with the ACE inhibitor, perindopril for 20 weeks, had beneficial effects on muscle performance, although the mechanism is unknown (Witham, Sumukadas, & McMurdo, 2008). Thus, besides having a favorable amino acid composition, meat may also contain bioactive peptides capable of stimulating muscle accretion.

3. Protein hydrolysates

The cells lining the gastrointestinal (GI) tract exhibit tight junctions that are believed to be largely impermeable to intact proteins except in newborns, where functionally active proteins can be taken up. Proteins are therefore hydrolyzed extensively during GI transit, first by pepsin released in the gastric juice, then by pancreatic proteases released into the duodenum, and subsequently by peptidases, particularly dipeptidyl peptidase IV (DPP-IV), in the jejunum and ileum. Thus, converted to amino acids and small peptides, meat proteins are efficiently taken up by amino acid and peptide transporters situated on the surface of enterocytes in the GI and delivered to systemic circulation to serve as metabolic precursors for protein synthesis and other pathways. Whereas most protein functionalities are believed to be lost during acid denaturation and peptic cleavage in the stomach, potential functionality of peptides generated during GI digestion, so-called cryptic peptides (Meisel & FitzGerald, 2000), has spurred considerable scientific interest. This is particularly true for milk proteins (Park, 2009), where several peptides released during digestion of caseins have been ascribed potential biological functions, although mostly based on in vitro assays. Meat protein hydrolysates, dominated by peptides derived from myosin and actin, have also been the subject of interest for potential bioactivity (Ryan, Ross, Bolton, Fitzgerald, & Stanton, 2011). There already exist excellent and comprehensive reviews on the subject of bioactive meat protein hydrolysates (e.g. Arihara, 2006; Arihara & Ohata, 2008; Kovacs-nolan & Mine, 2010), and in the following we will only highlight a few selected areas that we find particularly interesting.

Meat protein hydrolysates, obtained either as i) autolysates by endogenous protease activities, or ii) from controlled digestion with added proteases, mostly of bacterial or fungal origin, or iii) by microbial fermentation, have traditionally been utilized as feed additives for livestock animals, but little for human consumption, except maybe fermented fish sauce. Shortage of meat supplies, waste concerns, or indications of beneficial physiologic activities have, however, instigated exploitation of the potential of meat protein hydrolysates as food or food ingredients.

Meat hydrolysates (Ahmed & Muguruma, 2010; Vercruyssen, Van Camp, & Smagghe, 2005) as well as fish protein hydrolysates have been ascribed many potential bioactivities (Chalamiaiah, Dinesh kumar, Hemalatha, & Jyothirmayi, 2012; Fitzgerald et al., 2005), particularly in regulation of hypertension due to ACE inhibition (Ryan et al., 2011). However, other positive effects, e.g. reduction of visceral fat deposition, and possible mechanisms for this effect have been proposed for protein hydrolysates, such as regulation of bile acid metabolism (Liaset et al., 2009; Liaset et al., 2011), as well as induction of satiety (Cudennec, Fouchereau-Peron, Ferry, Duclos, & Ravallec, 2012).
3.1. Physiologic activities of meat protein hydrolysates in GI

3.1.1. Satiety

A series of seminal papers have addressed the satiating effect of dietary compounds by duodenal infusion in rats, where glucose (Wolman & Reidelberger, 1996), fatty acids (Wolman, Castellanos, & Reidelberger, 1995), and meat protein hydrolysates (Wolman & Reidelberger, 1999) clearly affected the release of cholecystokinin (CCK), a major gut-produced peptide hormone that induces satiety (Steinert & Beglinger, 2011), and resulted in lower dietary intake due to smaller and less frequent meals. Involvement of CCK-mediated signaling was demonstrated with the CCK receptor antagonist devazepide, although subsequent work (Pupovac & Anderson, 2002; Trigazis, Oettmann, & Anderson, 1997; Trigazis, Vaccarino, Greenwood, & Anderson, 1999) indicated that other signaling pathways via opioid receptors were also involved. Satiety inducing effects were obtained with both animal (casein) and plant-derived (soy) protein and these were greatest with hydrolysates. Thus, it appears that meat protein hydrolysates might induce the same signals as do vegetable protein hydrolysates. Recently, intragastric infusion in rats of a pea protein hydrolysate was shown to reduce subsequent meal size more than that obtained with intact pea protein (Häberer et al., 2011).

3.1.2. Amino acid and peptide sensing in GI is mediated by taste receptors

Gustatory functionality throughout the GI has bearing on comparative morphology between cells of the taste buds and those in GI (Fujita, 1991), as subsequently shown for glucose (Jang et al., 2007) and other nutrients (Yan & Pasricha, 2008).

Using established cell lines of enteroendocrine epithelial cells that respond to nutrients in GI, meat protein hydrolysates have been shown to stimulate specific signaling pathways (Reimer, 2006) that lead to improved insulin response through increased release of incretins such as glucagon-like-peptide 1 (GLP-1). This could be of considerable importance in conditions of decreased insulin sensitivity as is seen in metabolic syndrome, including obesity, diabetes II, and hypertension (Abhmed & Muguruma, 2010), because GLP-1 and other gut hormones reduce food intake and affect appetite regulation in the brain (De Silva et al., 2011). GLP-1 secretion is also caused by amino acids, particularly l-glutamine, acting through GPRC6A (G-protein coupled receptor family C group 6 member A) (Oya et al., 2013; Tolhurst et al., 2011). Peptide transporters (Liou et al., 2011) and taste receptors (Daly et al., 2013) expressed in the gut induce secretion of the satiety inducing hormone CCK (Steinert & Beglinger, 2011). The potential complexity of functional taste receptors, composed of G-protein coupled heterodimers, expressed in the gut is large (Reimann, Tolhurst, & Gribble, 2012) and nutrient stimuli signaling through these receptors is only beginning to be unraveled (Iwatsuki & Uneyama, 2012; Janssen & Depoortere, 2013). Possibly, the combined action or synergy of a number of different but concurrent stimuli from dietary meat or meat protein hydrolysates may prove effective in preventing or treating metabolic syndrome (Holst & McGill, 2012).

4. ACE-inhibitory components from connective tissue

Angiotensin I-Converting Enzyme (ACE) is part of the renin–angiotensin system (RAS), which play a key role in maintaining blood pressure homeostasis and fluid and salt balance as well as local tissue growth, remodeling and inflammation (Guang, Phillips, Jiang, & Milani, 2012). Muscle protein hydrolysates are a source of ACE inhibitors (Vercruysse et al., 2005). Peptides generated from enzymatic hydrolysis of the insoluble myofibrillar muscle protein fraction (reviewed by Vercruysse et al., 2005) as well as the sarcoplasmic muscle protein fraction (Jang & Lee, 2005) contains ACE-inhibitory activity. Collagen is a key component of meat, although mostly discussed for its contribution to toughness of meat and as such a low value component. However, a bioactive effect was shown by Saiga et al. (2003). This group showed an effect of chicken breast muscle extract on the blood pressure of spontaneously hypersensitive rats, which could be related to the generation of peptides from collagen after gastric hydrolysis. These peptides had ACE-inhibitory effect. ACE cleaves inactive angiotensin I (Ang-I), which is released from the liver as angiotensinogen, and converted to Ang-I by renin. The product of ACE hydrolysis is angiotensin II (Ang-II), which in effect causes an increased blood pressure (Guang et al., 2012). Thus, blocking the activity of ACE prevents the blood pressure induced by Ang-II. Synthetically produced ACE inhibitors are on the market, however Guang and Phillips (2009) suggest that food derived inhibitors might have more tissue affinity and be more slowly eliminated. Lately, Zhang et al. (2010) showed that long term feeding with chicken collagen hydrolysate to rats, which were fed N-nitro-l-arginine methyl ester (L-NAME) to induce systemic arterial hypertension, caused a suppression of the systolic blood pressure, an improvement of survival rate, and after eight weeks of treatment an inhibition of cardiovascular damage of the endothelial cells. The mechanism of anti-inflammatory action is suggested to be related to nitric oxide (NO). Zhang et al. (2010) found an increase in NO in serum of rats one hour after oral consumption of chicken collagen hydrolysate. NO inhibits expression of cell adhesion molecules in the vascular endothelium, which may prevent the cardiovascular damage to tissues and blood vessels (Zhang et al., 2010).

Kong et al. (2011) optimized the production of ACE-inhibitory peptides from collagen and obtained better inhibition with peptides generated by pepsin (78%) or pepsin and trypsin (88%), when compared to those generated by protease B from Bacillus polymyxa, protease M from Aspergillus oryzae and papain. This illustrates that the active peptides can be generated with gastric enzymes. What needs to be settled is the potential of collagen from other species.

Collagen hydrolysates are also potential therapeutic agents for osteoarthritis and osteoporosis (Deal & Moskowitz, 1999; Moskowitz, 2000). The mechanisms here are provision of building blocks required for renewing bone collagen, but lately actions on human fetal osteoblasts in vitro showed positive effects on cell viability, proliferation and an anti-apoptotic effect using gelatin hydrolysates prepared from chum salmon (Fu & Zhao, 2013). These mechanisms can most probably be coupled with specific peptides generated during hydrolysis, but more work is needed to pin-point the most active peptides.

5. Nucleotides and nucleosides

Humans and other mammals are able to synthesize nucleotides de novo, by an energy requiring process, or via the salvage pathway (Walker, 1994), but as enterocytes along with brain cells and bone marrow cells have limited de novo synthesizing capacity (Yamamoto, Wang, Adjie, & Ameho, 1997), these cells are dependent on supplements from dietary sources. Accordingly, dietary nucleotides are deemed conditionally essential in the presence of various physiological stresses, including growth and development, recovery from injury, infection, and certain disease states (Hess & Greenberg, 2012). For dietary supplement meat is a good source especially during periods of stress, where tissue requirement for nucleotides is increased (Carver & Walker, 1995) and nucleotides are considered conditional essential nutrients for infants (Uauy, Quan, & Gil, 1994). Most dietary nucleotides are absorbed as nucleosides and subsequently phosphorylated in the enterocyte. This is a relatively efficient process since more than 95% of dietary nucleotides are absorbed (Uauy et al., 1994). However, most of the purines are metabolized to uric acid in the enterocytes and only small quantities appear in the hepatic cells. Therefore, ingestion of nucleotides is followed by an increase in the expression and activity of enzymes involved in nucleotide metabolism. In contrast to the purines, relatively large quantities of dietary pyrimidines are transported from the enterocytes to the hepatic portal vein (Uauy et al., 1994).
Due to the limited capacity of the enterocytes for nucleotide synthesis and because nucleotides are needed in cell synthesis, dietary nucleotides enhance the growth, differentiation and maturation of intestinal epithelial cells (Rodriguez-Serrano et al., 2010; Sanderson & He, 1994). It has been shown that dietary nucleotides may increase the formation of mucosal protein, the concentration of DNA, and the length of the villi in the small intestine (Carver & Walker, 1995; Uay, Stringel, Thomas, & Quan, 1990).

Both dietary and parenteral supplementation of nucleic acids support mucosal cell proliferation and function as demonstrated by increased mucosal weight, protein and DNA contents, villous height, and narrower tight junctions of the jejunal mucosa (Dancey, Attree, & Brown, 2006; Kishibuchi et al., 1997; Tsuchinaka, Kishibuchi, Lijima, Yano, & Monden, 1999).

Nucleotides in the diet are reported in some cases to affect elongation and desaturation of long-chain fatty acids, which might have an impact on infant nutrition (Gibson, Hawkes, & Makrides, 2005; Gil et al., 1988).

During exercise and physical training, dietary nucleotides can be supportive of the immune system, decrease cortisol levels and stress symptoms, and be supportive of the immune system (Mc Naughton, Bentley, & Koeppel, 2006; Mc Naughton, Bentley, & Koeppel, 2007).

Finally, there is an emerging and very exciting area of research of the epigenetic influence of dietary microRNAs on host gene expression and metabolism, both in animal models and humans (Zhang et al., 2012). So far there are only reports for plant constituents, which are shown to inhibit e.g. tumor development, both directly as microRNA (Parasramka et al., 2012), or indirectly affecting host microRNA (Dhar, Hicks, & Levenson, 2011). But it would be very interesting to investigate the effects of meat microRNA from this perspective.

6. Phytanic acid

The fatty acid (FA) profile of meat from monogastric animals is to a large extent a reflection of the composition of fatty acids in the feed. In contrast, FA composition of meat from ruminants is only to some extent affected by feeding because unsaturated FAs stemming from plant material are fully or partly hydrogenated by microbial processes in the rumen. Further, several FAs are synthesized by the ruminal microorganisms. One of the FAs characteristic of ruminal products is phytanic acid (3,7,11,15-tetramethyl hexanoic acid). This saturated branched FA is synthesized from phytol, which is cleaved from chlorophyll and subsequently oxidized and hydrogenated to form phytanic acid (Ackman & Hansen, 1967; Patton & Benson, 1966). This makes ruminant products a vital source of phytanic acid and as the primary substrate is chlorophyll, green feed items are essential for the formation of phytanic acid. Data on the phytanic acid content of meat are very scarce but available estimates suggest that it may vary from 4 mg/100 g in lean organic beef to over 30 mg/100 g in organic beef fat (Brown et al., 1993). More information is available on the phytanic acid content of milk (Che, Kristensen, et al., 2013; Che, Oksbjerg, et al., 2013) and indications lead to potentially similar implications for the impact on feeding on phytanic acid content of milk (Che, Kristensen, et al., 2013; Che, Oksbjerg, et al., 2013). This makes FA is synthesized from phytol, which is cleaved from chlorophyll in the rumen. Further, several FAs are synthesized by the ruminal microbial processes, and the CLA content has been shown to increase during heating >80 °C (Dhiman et al., 2005). The concentration of phytanic acid in human plasma depends on the content of phytanic acid in the diet, and ranges from 0.486–5.77 μM, with vegans and meat-eaters representing the lowest and highest values, respectively (Allen et al., 2008). Since phytanic acid naturally exists in two enantiomeric forms; RRR and SRR, but available data on the physiological impact of phytanic acid has not distinguished between these isomers. Although no difference between the isomeric forms on agonist activity towards PPAR α has been shown (Heim et al., 2002), there are pertinent reasons to believe that the biological impact of the isomeric forms differ. Differential occurrence of the isomeric forms in lipid fractions in serum implies different preferences for enzymes of the oxidation system (Eldjarn & Try, 1968). Furthermore, at higher phytanic acid concentrations, it has been shown that the SRR form is more rapidly oxidized in mitochondrial preparations (Tsai, Steinber, Avigan, & Fales, 1973). These findings make the distribution of the phytanic acid isomers in meat potentially relevant.

Alpha-methylacyl-CoA racemase (AMACR) is required for conversion of RRR to SRR-phytanic acid before utilization by beta-oxidation enzymes (Ferndandusse et al., 2002). As AMACR has been implicated in the occurrence of prostate cancer (Zheng et al., 2002), this draws concern to the content of the RRR form of phytanic acid in humans. Interestingly, phytanic acid has been shown to inhibit the proliferation of prostate carcinoma cells in culture (Tang, Suh, Li, & Gudas, 2006), and no overall effect of AMACR has been linked to phytanic acid (Hellgren, 2010).

7. Conjugated linoleic acids

Conjugated linoleic acids (CLA) are a group of positional and geometric isomers of octadecadienoic acid found in meat and milk of ruminants, which have initially been identified as anti-carcinogenic compounds in the extracts of grilled beef (Schmid, Collomb, Sieber, & Bee, 2006). Several CLA isomers are formed from linoleic acid by rumen bacterial isomerases, however, the main CLA in meat, C18:2 cis9,trans11, is to a wide extent formed by desaturation of vaccenic acid (C18:1 trans11) in the adipose tissue (Schmid et al., 2006). In addition to its anti-carcinogenic property, CLA has anti-atherosclerotic, antioxidative, and immunomodulatory properties (Azain, 2003). CLA may also play a role in the control of obesity, reduction of the risk of diabetes and modulation of bone metabolism. However, most effects have been reported from in vitro studies or from studies in rodents. Corresponding and persistent effects in humans remain to be shown. The CLA content of milk has been widely studied and varies according to breed, management, feed composition and stage of lactation. Typical values for the CLA content of milk range from 4 to 20 mg/g milk fat (Butler et al., 2011). The CLA content in meat has attracted less interest, but is affected by similar factors as the CLA content of milk (Dhiman, Nam, & Ure, 2005). Mean CLA content in beef between 1 and 10 mg/g fat has been reported (Schmid et al., 2006). A comparison of feeding a mix of barley and silage to entirely pasture has shown an increase in the CLA content of meat from 3 to 14 mg/g fat (Poulos, Dhiman, Uh, Cornforth, & Olson, 2004). Besides, the CLA content may increase during heating >80 °C (Dhiman et al., 2005).
8. Antioxidants

Several endogenous antioxidants, e.g. glutathione, uric acid, spermine, carnosine, and anserine, that are characteristic for meat have been studied (Decker, Livisay, & Zhou, 2000). Both carnosine (\(\beta\)-alanyl-L-histidine) and anserine (N-(\(\beta\)-alanyl-1-methyl-L-histidine) are antioxidative histidyl dipeptides and the most abundant antioxidants in meat (Guiotto, Calderan, Ruzza, & Borin, 2005). The concentration of carnosine in meat ranges from 500 mg/kg of chicken thigh to 2700 mg/kg of pork shoulder (Purchas & Busboom, 2005; Purchas, Rutherford, Pearce, Vather, & Wilkinson, 2004). Anserine is especially found in beef and seems to be related to stress. The antioxidative activity of these dipeptides may result from their ability to chelate transition metals (Brown, 1981) and form complexes with e.g. copper, zinc and cobalt. Depending on the metal ion bound, the complexes display different biological functions (Baran, 2000), for example the carnosine zinc complex alleviates injuries to the gastric mucosa, acts against stomach ulcers and inhibits the major pathogen Helicobacter pylori (Matsukura & Tanaka, 2000). These antioxidative peptides have also been reported to play a role in wound healing, recovery from fatigue and prevention of diseases related to stress.

Carnosine and anserine are only found in meat, poultry and some fish, but not in foods of plant origin. The bioavailability of carnosine from meat has been demonstrated by measuring the concentration in human plasma after consumption of 200 g minced beef, reaching its highest level (32.7 mg/l) after 2.5 h, and after 5.5 h no more carnosine could be detected (Park, Volpe, & Decker, 2005).

9. In vitro meat production and nutritional design

9.1. Production

The human population will increase to 9 billion people in 2050, and FAO estimates a doubling of current meat consumption, in part also due to increased living standards. Current meat production has severe impact on our environment, including emission of CO\(_2\) and high toll on fresh water supply and arable land. Consumers are aware of animal welfare problems also. A more sustainable route than simple expansion of current practices for meat production is production of meat without animals (Meisel & FitzGerald, 2000). Because traditional meat production contributes 18% of total CO\(_2\) emission, switching to \textit{in vitro} meat may reduce this emission to less than 1% (Tuomisto & Teixeira de Mattos, 2011). Making arable land available for cultivation of vegetable food rather than meat production will further improve global climate. Thus, \textit{in vitro} meat may aid both our climate and secure meat as a more sustainable technological alternative in the future (http://www.un.org/en/sustainablefuture). Economically, global cost savings of replacing traditional meat with that produced \textit{in vitro} could be more than $130 billion per year (http://www.new-harvest.org/resources.htm).

\textit{In vitro} reproduction of muscle requires stem cells that divide and differentiate into mature muscle fibers. For these processes to be efficient, the cells need solid substrates for proliferation as well as specific growth factors. Furthermore, high cell densities require very efficient bioreactors for adequate nutrient supply and waste removal. Thus, there are a number of scientific and technological challenges that must be addressed before efficient cultivation of muscle cells can be achieved. First, appropriate cells capable of proliferation and differentiation must be selected and cost-effective growth media developed. Second, food-compatible and edible substrates necessary for muscle cell attachment, growth and maturation must be developed and should also contribute to the texture of \textit{in vitro} meat. Third, production must be scalable for industrial production. Fourth, high nutritional value and consumer acceptance of novel products containing \textit{in vitro} meat must be attained.

9.2. From cell culture to meat

9.2.1. Choice of cell type

Cultivation of muscle \textit{in vitro} based on embryonic stem cells (ESC) and satellite cells (SC) has been described (Edelman, McFarland, Mironov, & Matheny, 2005; Nissen & Oksbjerg, 2009; Wilschut, Jakansi, Van Den Dolder, Haagsman, & Roelen, 2008). ESC can in principle divide indefinitely and differentiate into all cell types, but until now this has only been possible with cells from rodents, rhesus monkeys and humans. Alternatively, SC isolated from muscle of live-stock animals have limited reproduction potential with up to 120 population doublings (Wilschut et al., 2008) and require an extracellular matrix or scaffold for adherence and growth (Wilschut, Haagsman, & Roelen, 2010), but given appropriate stimuli can fuse into myotubes (Theil, Sorensen, Therkildsen, & Oksbjerg, 2006).

9.2.2. Scaffold

Recently, the potential for edible and biodegradable electrospun polymers was demonstrated for muscle tissue engineering, where electrospun polymers produced at high speed resulted in fibers that were aligned in parallel like muscle fibers thus allowing muscle cells to adhere, elongate and differentiate in parallel bundles (Aviss, Gough, & Downes, 2010). Because muscle cells are adherent cells and cannot grow in suspension, SCs can be seeded on edible porous microspheres in suspension making a huge increase in surface area and allowing high cell densities (>10^6 cells/ml).

9.2.3. Maturation

Exercising myotubes to stimulate accretion of contractile proteins (myosin and actin) is necessary and possible with low-field electrical pulses and mechano-adherent stretching using temperature-sensitive hydrogels and/or flexible nanofibers to simulate exercise of muscles in vivo (Boonen, van der Schaft, Baaijens, & Post, 2011; Langelaan et al., 2011; Powell, Smiley, Mills, & Vandenhurk, 2002). The growth medium for cultivation of muscle cells must contain glucose, amino acids, vitamins, minerals and growth factors. Whereas glucose, vitamins, amino acids and minerals can be supplied from plants or other sustainable sources, e.g. blue-green algae or algae, specific growth factors must be produced by bacteria or plants by recombinant means.

9.2.4. Nutritional value

Meat has high nutritional value when it comes to amino acid composition, B12 vitamin, and highly available iron. Further, during \textit{in vitro} growth of muscle cells it will be possible to enhance various nutritious factors, e.g. the amount of \(\omega-3\) fatty acids through supplementation directly in the growth medium. \textit{In vitro} meat may consequently end up being a healthier product than meat from animals. \textit{In vitro} meat does not contain significant amounts of lipids necessary for good eating quality and meat taste, except for a small amount of intracellular and membrane bound phospholipids. Unlike \textit{in vivo} produced meat \textit{in vitro} meat will not possess intra muscular fat between fibers. However, co-culturing with adipocytes may be a potential solution. Similarly, additional texture of the \textit{in vitro} grown muscle cells may be obtained by co-culture with fibroblasts, which produce collagen. Novel food products produced \textit{in vitro} from animal stem cells will require approval by national food authorities, and regulations will eventually have to be developed by governmental regulatory agencies. In principle, production of \textit{in vitro} meat could be carried out with stem cells from various species including animals not currently used for meat production. Further, European consumers in particular may also consider the use of stem cells or genetically modified cells from meat animals a problem. Although the concept of \textit{in vitro} meat production is not new, research and development in this area are needed to bring new products to the consumer. We believe a combination of expertise from several disciplines will lead to novel products of edible hydrogels, design of new and efficient
10. Conclusion
Meat provides many functional ingredients of importance for health, and more may be described in the future as our understanding of the interaction between health and diet continues to improve and unfold. We have described how peptides and specific amino acids may play a role for muscle accretion especially in relation to muscle wasting diseases, and possible mechanisms behind satiety induction by protein hydrolysates with implications for welfare diseases. Theories on the ACE-inhibitory effect of degradation products of connective tissue have been put forward as well as the fundamental role of nucleotides for development and maintenance of the intestinal tract. Phytanic acid as a hitherto overlooked meat component has been described as a ligand of PPAR, but also the slightly better researched impact of CLA and meat specific antioxidants and their consequent health improving properties have been reviewed. Finally, the options, challenges and perspectives of in vitro meat production have been outlined together with possible alternative methods of influencing the nutritional impact of designed in vitro meat. We find these novel or rediscoved features of meat both fascinating and challenging for future research and of potential commercial importance.

References


