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Peptides reproducibly released by *in vivo* digestion of beef meat and trout flesh in pigs

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Characterisation and identification of peptides (800 to 5000 Da) generated by intestinal digestion of fish or meat were performed using MS analyses (matrix-assisted laser desorption ionisation time of flight and nano-liquid chromatography electrospray-ionisation ion trap MS/MS). Four pigs fitted with cannulas at the duodenum and jejunum received a meal exclusively made of cooked *Pectoralis profundus* beef meat or cooked trout fillets. A protein-free meal, made of free amino acids, starch and fat, was used to identify peptides of endogenous origin. Peptides reproducibly detected in digesta (i.e. from at least three pigs) were evidenced predominantly in the first 3 h after the meal. In the duodenum, most of the fish- and meat-derived peptides were characteristic of a peptic digestion. In the jejunum, the majority of peptides appeared to result from digestion by chymotrypsin and trypsin. Despite slight differences in gastric emptying kinetics and overall peptide production, possibly in relation to food structure and texture, six and four similar peptides were released after ingestion of fish or meat in the duodenum and jejunum. A total of twenty-six different peptides were identified in digesta. All were fragments of major structural (actin, myosin) or sarcoplasmic (creatine kinase, glyceraldehyde-3-phosphate dehydrogenase and myoglobin) muscle proteins. Peptides were short (< 2000 Da) and particularly rich in proline residues. Nineteen of them contained bioactive sequences corresponding mainly to an antihypertensive activity. The present work showed that after fish or meat ingestion, among the wide variety of peptides produced by enzymic digestion, some of them can be reproducibly observed in intestinal digesta.

Peptides: Meat: Fish: Digestion: Mass spectrometry

The concept of food-derived peptides possessing biological activity, or bioactive peptides, brings a completely new dimension that should be considered in the description of dietary protein quality. Bioactive peptides have been defined as protein fragments with specific sequences that have, in addition to their nutritional value, a positive impact on body functions and may ultimately influence health[1]. Once liberated in the body, they can exert different beneficial effects such as antihypertensive, antithrombotic, opioid, immunomodulating, antimicrobial and antioxidant activities[1,2]. Most dietary proteins contain bioactive sequences, but the corresponding peptides need to be released by proteolysis, during food processing or gastrointestinal digestion, in order to show a biological activity.

Many bioactive peptides have been identified in milk products[3,4]; in comparison, less information is available for meat and fish products. Such products may nonetheless represent a valuable source of peptides with biological activities[5,6]. For example, two angiotensin-converting enzyme (ACE) inhibitory peptides, obtained by *in vitro* thermolysin digestion of porcine skeletal muscle[7], were shown to have an *in vivo* antihypertensive activity when administered orally to spontaneously hypertensive rats[8]. However, there is evidence that peptides produced by *in vitro* digestion yield different peptides compared with those generated *in vivo*. For example, β-casomorphin fragments isolated from intestinal contents following ingestion of casein[9] are larger fragments than those found in *in vitro* digesta. To our knowledge, studies on the occurrence of bioactive peptides in digesta after ingestion of meat or fish have not been reported. More generally, and despite the central role of digestive events in the supply of nutrients or biologically active compounds to the organism, almost no information is available on peptides resulting from *in vivo* digestion of meat or fish, in particular their sizes and sequences, let alone the random or reproducible manner in which they are generated.

In this context, and following description of peptides present in ready-to-eat beef meat or trout[10,11], the present study aimed at characterising and identifying peptides (< 5 kDa) generated by intestinal digestion of such food products, using the pig as an animal model for man.

Abbreviations: ACE, angiotensin-converting enzyme; MALDI-ToF, matrix-assisted laser desorption ionisation time of flight; nano-LC-ESI-IT MS/MS, nano-liquid chromatography electrospray-ionisation ion trap MS/MS; PP1, first 3 h period after meal; PP2, second 3 h period after meal.

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Materials and methods

Animals

The study involved four 3-month-old female pigs (40·5 (SD 5·2) kg). At 3 weeks before the experimentation, pigs were surgically fitted with T-shaped cannulas (silicone rubber; 12 mm internal diameter, 17 mm outer diameter) in the duodenum (10 cm downstream from the pylorus) and the mid-jejunum (about 4 m downstream from the pylorus), and a catheter (polyvinyl chloride; 1·1 mm internal diameter, 1·9 mm outer diameter) in the cava vein. Surgical procedures and post-surgical care were conducted in accordance with national legislation on the care and use of laboratory animals. Pigs were housed in individual pens (1 × 1·5 m) in a ventilated room with controlled temperature (19–23°C). They were given 800 g/d of a concentrate feed containing 18 % protein, 2 % fat, 5 % cellulose and 6 % ash (Porcyprima; Sanders Centre Auvergne, Aigueperse, France) distributed in two equal portions at 8.00 and 16.00 hours, and had free access to water. The daily ration was adjusted to cover 150 % of the maintenance requirements[12].

Test meals

Three types of foods (beef, trout, protein-free food) were prepared. Beef meat was *Pectoralis profundus* from a 15-month-old Charolais bull, aged for 14 d, and cooked under vacuum for 90 min at 75°C in a water-bath. Trout was the dorsal part of rainbow trout (*Oncorhynchus mykiss*) fillets, stored for 7 d on ice, and cooked under-vacuum for 5 min at 70°C in a water-bath. Both types of muscle foods were frozen at −20°C until use. The protein-free food, used for the purpose of monitoring the endogenous peptide composition of digesta in a postprandial situation, was made of 47 % free amino acids (in proportion close to the amino acid composition of muscle), 23 % wheat starch, 12 % fat and 18 % water. Each test meal provided 40 g crude protein, or the equivalent in amino acids, and was exclusively made of either beef (115 g; 44 g DM), trout (174 g; 44 g DM) or the control protein-free food mixture (85 g; 75 g DM). Meals were offered for 15 min and were always consumed entirely within that time.

Experimental procedures

The three types of foods were tested on each animal. Test meals were separated by at least 2 d. Duodenum and jejunum digesta sampling was performed on two distinct test meals. The test meal was given at 09.00 hours. Digesta were continuously collected from 08.00 to 15.00 hours in cold plastic bottles replaced each hour. Water intake was restricted to 250 ml/h. To prevent dehydration, 1 litre Ringer-lactate solution was intravenously infused at a rate of 150 ml/h throughout the sampling session.

Hourly collected digesta were immediately homogenised in a Waring Blender (VWR International SAS, Fontenay-sous-Bois, France) (twice 5 s at high speed, separated by 5 s). A first fraction of the homogenate (about 10 g) was used for DM determination (24 h at 104°C). A second fraction (about 100 g) was treated with perchloric acid at 170 mM (final concentration). After vigorous shaking, samples were kept on ice for 15 min, and then centrifuged at 10 000 ***g*** for 20 min at 4°C. For the postprandial period, fractions of supernatant fractions (2 % of hourly collected digesta) were pooled to yield two samples per animal: a first pool representative of the 3 h following the meal (PP1), and a second one representative of the period spanning from 3 to 6 h after the meal (PP2). Post-absorptive (sample collected before the meal), PP1 and PP2 supernatant fractions were filtered through 5000 Da cut-off filters (Vivaspin 15; Vivascience, Hanover, Germany) at 2000 ***g*** for 8 to 15 h at 4°C. Filtrates were stored at −80°C until analysis.

Analytical methods

Chromatographic analysis of peptides in extracts was carried out using an HPLC system (Kontron Instruments, Bletchley, Bucks, UK). Separation was done in a Nucleodur® C18 pyramid (110 Å; 4·6 × 250 mm) with 5 μm particles (Macherey-Nagel, Düren, Germany) at 30°C in a column oven and at a flow rate of 0·9 ml/min. The gradient was performed using two solvents (A, 0·1 % trifluoroacetic acid in water; B, 0·1 % trifluoroacetic acid in 100 % acetonitrile) and formed as follows: 0 % B up to 5 min, 0–60 % B in 5–35 min and 60–100 % B in 35–38 min. Detection of peptide bonds was done at 220 nm.

Matrix-assisted laser desorption ionisation time-of-flight (MALDI-ToF) analysis and nano-liquid chromatography electrospray-ionisation ion trap MS/MS (nano-LC-ESI-IT MS/MS) analysis were performed as previously described by Bauchart *et al.*[10]. For MALDI-ToF analysis, masses were recorded in the *m/z* range 800 to 5000. Below 800, *m/z* may not be reliably monitored by MALDI-ToF because of interference with matrix-generated peaks.

Statistical analyses

DM flux, at each site, during the first 3 h after feeding (PP1) was examined by a two-way randomised-block ANOVA with food as factor and animal as block using SAS version 8.10 (SAS Institute Inc., Cary, NC, USA).

A statistical procedure, previously described[10], was applied to MALDI-ToF data to determine whether peaks, at close *m/z* but originating from different spectra, were likely to correspond to the same compound. For one type of food and one sampling time (PP1, PP2), only the peaks gathered in a group consisting of data originating from at least three animals were considered. A similar procedure was subsequently applied to determine which groups of peaks corresponded to the same compound, across sampling times.

Results

Intestinal transit kinetics

DM collected at the duodenum after ingestion of protein-free food, trout or beef during the three first 3 h after feeding (PP1) accounted for 88·5 (SD 4·0), 90 (SD 1·2) and 74 (SD 10·2) % of the total DM collected in the whole postprandial period (data not shown). This proportion was lower for beef than for protein-free food and trout ($P<0·05$). At the jejunum level, the proportion of DM collected in PP1 was not affected by test-meal composition, and averaged 77 (SD 14) % (data not shown).

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Chromatographic profiles of digesta

Reverse-phase HPLC analysis of digesta extracts collected after the ingestion of protein-free food (Fig. 1(a) and (b)) revealed the presence of numerous endogenous peptides. Chromatographic profiles showed very few qualitative differences between duodenum and jejunum, with common peaks at retention times of about 5·0, 6·2, 8·3, 9·9, 15·3, 18·0, 19·5, 21·3, 23·4, 23·9, 24·2, 24·5, 24·9 and 27·4 min. These endogenous peptides were still observed after ingestion of trout (Fig. 1(c) and (d)) or beef (Fig. 1(e) and (f)) but

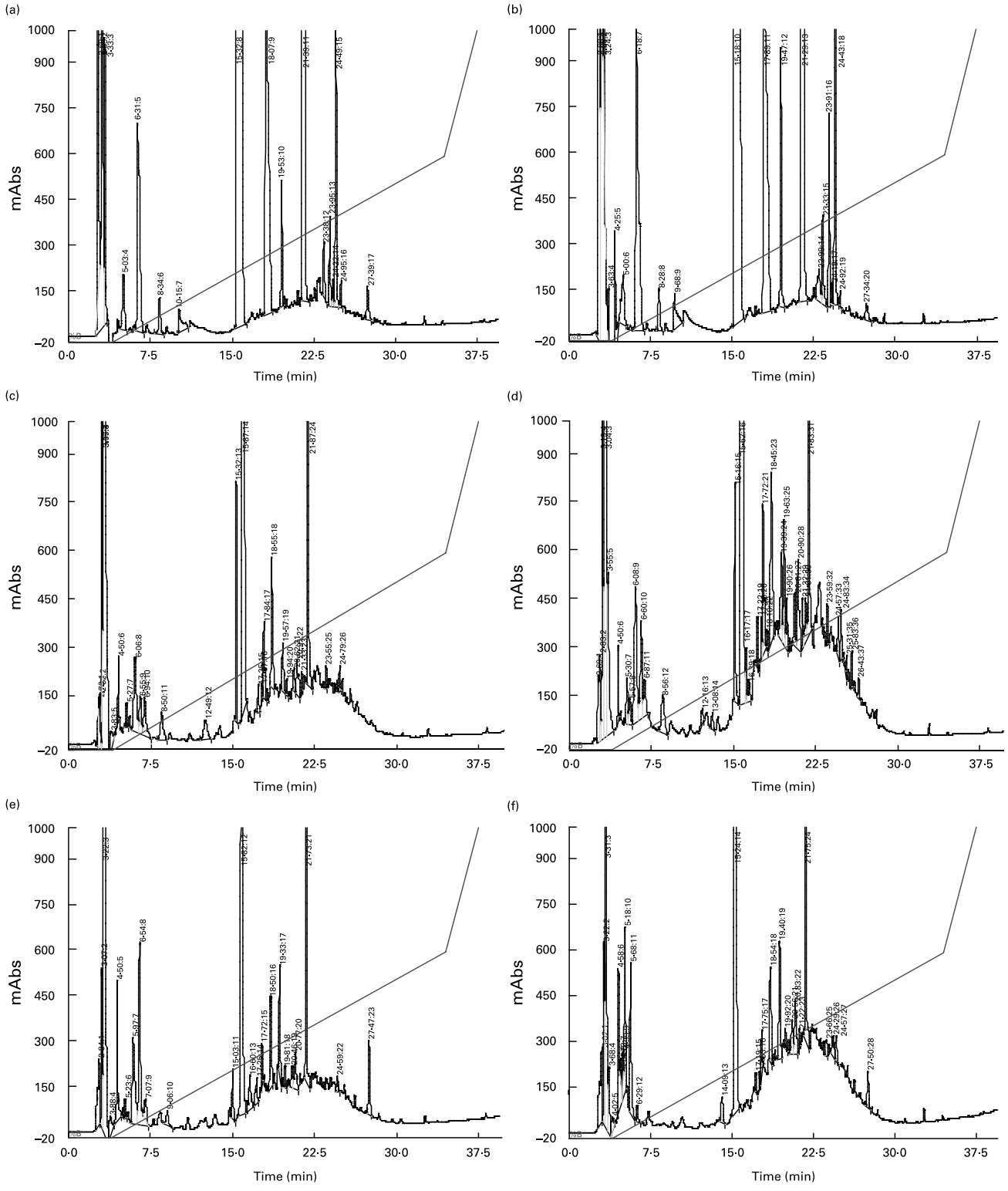

Fig. 1. Reverse-phase HPLC chromatograms of digesta extracts from the duodenum (a, c and e) and the jejunum (b, d and f) collected in one pig over the first 3 h following ingestion of control protein-free food (a and b), trout (c and d) and beef (e and f).

in addition, absorbance at 220 nm globally increased across the retention times 15 to 25 min. This increase was more prominent for jejunum digesta than for duodenum digesta, and generally larger after a meal of trout than after a meal of beef.

Peptides evidenced by matrix-assisted laser desorption ionisation time of flight analysis

Although peptides up to 5000 Da should be extracted by the procedure we used (perchloric acid precipitation of proteins and ultrafiltration), MALDI-ToF analysis evidenced very few compounds between 2000 and 5000 *m/z* in the digesta (Fig. 2). Masses of peptides detected by MALDI-ToF in at least three of the four pigs (later called 'reproducibly' detected) in digesta extracts after ingestion of beef or trout are listed in Table 1. All endogenous compounds, i.e. detected at least once in the intestinal contents of the post-absorptive period or after a protein-free meal, were removed from the list in order to retain only peptides of an alimentary origin. The time of sampling had a major effect on the number of

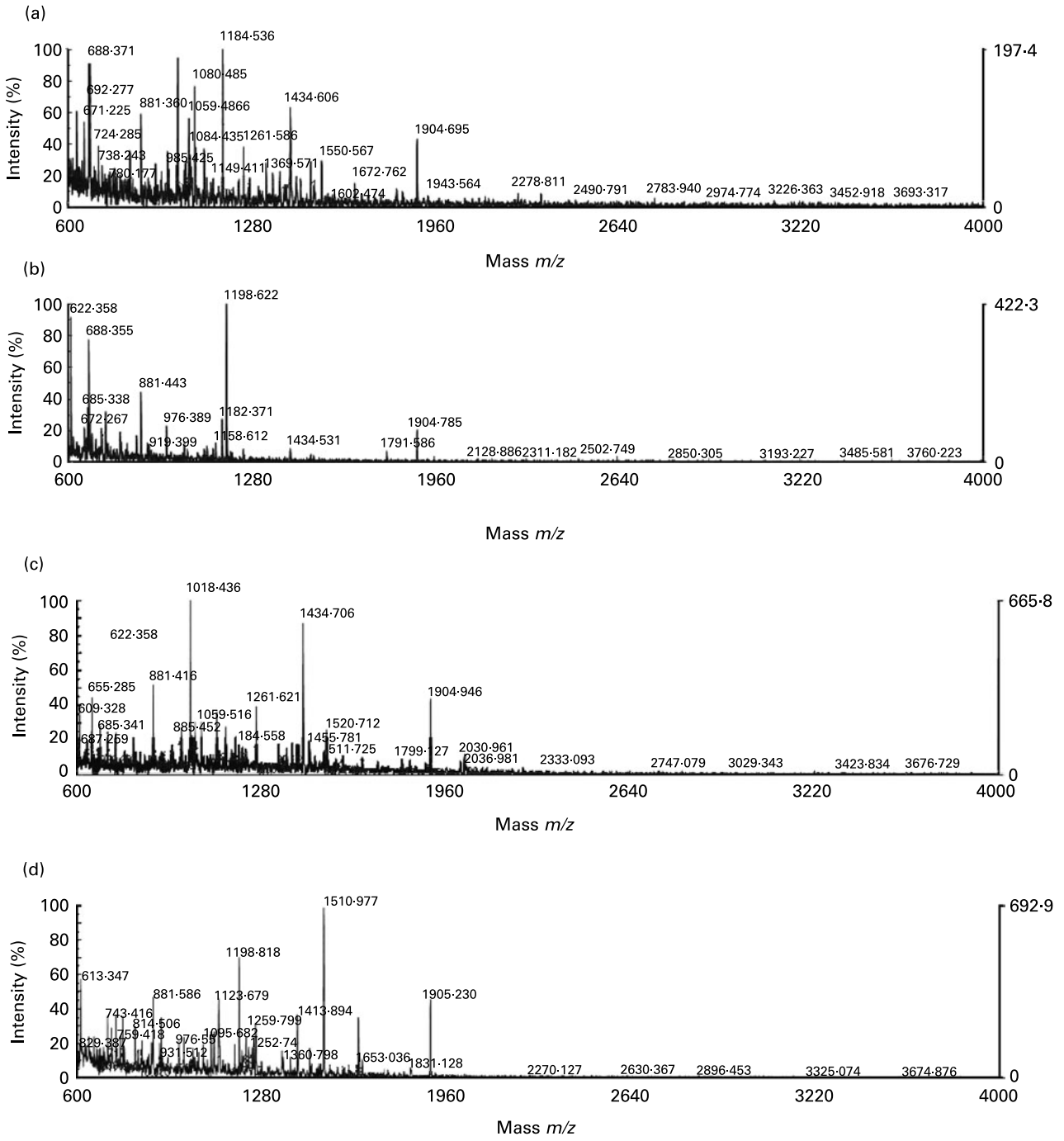

Fig. 2. Representative matrix-assisted laser desorption ionisation time of flight spectra of duodenum (a and c) and jejunum (b and d) digesta collected from one pig during the first 3 h after ingestion of cooked trout flesh (a and b) or cooked beef *Pectoralis profundus* (c and d).

reproducibly detected peptides, since in the second 3 h period after the meal (PP2) only one peptide (*m/z* 1018·50) was listed, in the duodenum after ingestion of beef. Compounds listed in PP1 were more numerous in the duodenum than in jejunum (eight and seven for trout; nine and five for beef). Some food-derived peptides were common to the duodenum and jejunum digesta, four for trout (*m/z* 1115·89, 1148·44, 1182·44 and 1434·17) and two for beef (*m/z* 1115·89 and 1198·75). In PP1, there was a striking similarity in the masses of peptides reproducibly generated by digestion of either trout or beef. At the duodenum level, out of eight and nine reproducibly detected peptides, seven were common to trout and beef. At the jejunum level, five were common to trout and beef out of seven and five.

Identification of reproducibly generated food-derived peptides

Fish- and meat-derived peptides identified by nano-LC-ESI-IT MS/MS analysis in digesta extracts collected over PP1 are listed in Table 2. The identified peptides came from abundant muscle proteins, namely actin, myosins, creatine kinase and glyceraldehyde 3-phosphate dehydrogenase, and myoglobin specifically for beef. Again, there were some similarities between the intestinal contents after a meal of trout or beef, with six and four food-derived peptides common to the two types of muscle foods in the duodenum and jejunum, respectively. Overall, a particularity of the sequences was the high proportion of proline, accounting for approximately 12·0 % (fifteen of 125) and 13·1 % (fourteen of 107) of all residues in peptides identified at the duodenum level for trout and beef, respectively. At the jejunum level, the proportion was even higher and reached 15·0 % (twelve of eighty) and 19 % (eleven of fifty-eight) for trout and beef, respectively. After the protein-free meal (data not shown) several fragments of pepsin A, trypsin precursor, pancreatic α-amylase and salivary proline-rich proteins were identified in duodenum digesta. Fragments of trypsin (IKLSSPATL and LIKLSSPATL) and salivary proline-rich protein (ARPLPGPPPPGPPP) were common to duodenum and jejunum digesta. The jejunum content was also characterised by the presence of many more fragments of salivary proline-rich proteins.

A search of potential biological activity of identified peptides was carried out with the BioPep database[13] and by comparison with sequences either reviewed in Vercruysse *et al.*[6] or described in Sentandreu & Toldrá[14]. Some sequences with potential biological activity were found within nineteen of the identified peptide sequences (Table 3). They were mainly short sequences of two or three residues, except for the sequences IVGRPR, YALPHA and ALPHA, all contained in actin fragments. The most frequently found potential biological activity was an antihypertensive effect.

Discussion

The present study intended to characterise and identify peptides reproducibly generated by *in vivo* gastroduodenal digestion of fish or meat, focusing on peptides ranging from 800 to 5000 Da. The lower limit was dictated by technical considerations while the upper limit was chosen in relation to the generally reported size of bioactive peptides. Overall, we have identified nineteen and fifteen different food-derived peptides in digestive contents of pigs after a meal of fish or beef, respectively. Many of the identified peptides contained bioactive sequences.

DM flux measured at the duodenal level illustrates the rapidity of gastric emptying after the meal, even more for trout than for beef. Food texture has been recognised as a factor influencing gastric emptying[15]. Here, in the cooking conditions used for beef, meat was dry and compact. The looser structure and moister texture of trout is a plausible explanation for the accelerated kinetics. Compositional factors, in particular the high proportion of *n*-3 PUFA in trout, may also contribute to a faster gastric emptying as described by Robertson *et al.*[16]. Values in the jejunum also evidence that transit throughout the anterior intestine proceeded rapidly.

After a meal of fish or meat, only one peptide was reproducibly detected in PP2 digesta, suggesting that peptides earlier generated by digestion were rapidly further processed by

Table 1. List of compounds (*m/z* 800 to 5000) detected by matrix-assisted laser desorption ionisation time of flight analysis in pig duodenum and jejunum digesta, collected during the first 3 h (PP1) and the subsequent 3 h (PP2) after ingestion of trout or beef*

Duodenum				Jejunum			
PP1		PP2		PP1		PP2	
Trout	Beef	Trout	Beef	Trout	Beef	Trout	Beef
				976·50	976·50		
1018·45	1018·45		1018·45				
	1035·34						
1115·89	1115·89			1115·89	1115·89		
1148·44	1148·44			1148·44			
				1158·59	1158·59		
1182·44				1182·44	1182·44		
1184·41	1184·41						
	1198·75			1198·75	1198·75		
1261·70	1261·70						
1434·17	1434·17			1434·17			
1520·77	1520·77						

* Listed compounds had an alimentary origin and were found in at least three of the four animals.

Table 2. Peptides identified by nano-liquid chromatography-ion trap tandem mass spectrometry in duodenum and jejunum digesta extracts collected in pigs over the first 3 h following ingestion of trout or beef*

Protein fragment	m/z ($M + H^+$)	Sequence	Accession number of the parent protein
Trout – duodenum			
Actin, skeletal muscle f(24–33)	1018·50	AGDDAPRAVF	gi 10953948
Actin, skeletal muscle f(96–106)	1261·70	LRVAPEEHPTL	gi 10953948
Actin, skeletal muscle f(97–106)	1148·61	RVAPEEHPTL	gi 10953948
Actin, skeletal muscle f(171–178)	915·48	YALPHAIM	gi 10953948
Actin, skeletal muscle f(269–278)	1043·48	IGMESAGIHE	gi 10953948
Actin, skeletal muscle f(328–339)	1455·91	KIKIIAPPERKY	gi 10953948
Myosin heavy chain f(109–115)	985·51	YNLKERY	gi 21623523
Myosin heavy chain f(835–842)	1021·65	YFKIKPLL	gi 21623523
Myosin light chain 2 f(106–115)	1209·62	FKVLDPDATGF	gi 7960275
Myosin light chain 3 f(153–160)	928·56	FVKHVLSV	gi 14335431
Creatine kinase f(195–204)	1128·67	LFDKPVSPLL	gi 64315
GA3PDH f(232–241)	1115·62	FRVPTPNVSV	gi 15010816
GA3PDH f(245–254)	1163·64	TVRLEKPASY	gi 15010816
Trout – jejunum			
Actin, skeletal muscle f(21–30)	976·45	AGFAGDDAPR	gi 10953948
Actin, skeletal muscle f(31–41)	1198·71	AVFPSIVGRPR	gi 10953948
Actin, skeletal muscle f(34–41)	881·53	PSIVGRPR	gi 10953948
Actin, skeletal muscle f(96–105)	1148·61	LRVAPEEHPT	gi 10953948
Actin, skeletal muscle f(241–252)	1334·68	SYELPDGQVITI	gi 10953948
Myosin heavy chain f(1488–1497)	1190·53	NSYEEALDHL	gi 21623523
Creatine kinase f(195–203)	1015·58	LFDKPVSPL	gi 64315
GA3PDH f(232–241)	1115·62	FRVPTPNVSV	gi 15010816
Beef – duodenum			
Actin, skeletal muscle f(24–33)	1018·50	AGDDAPRAVF	gi 27819614
Actin, skeletal muscle f(31–41)	1198·71	AVFPSIVGRPR	gi 27819614
Actin, skeletal muscle f(96–106)	1261·70	LRVAPEEHPTL	gi 27819614
Actin, skeletal muscle f(96–105)	1148·61	LRVAPEEHPT	gi 27819614
Actin, skeletal muscle f(171–180)	1184·66	YALPHAIMRL	gi 27819614
Actin, skeletal muscle f(171–178)	915·48	YALPHAIM	gi 27819614
Actin, skeletal muscle f(181–191)	1251·62	DLAGRDLTDYL	gi 27819614
Myosin heavy chain polypeptide 4 f(326–333)	1021·65	YFKIKPLL	gi 76643989
Creatine kinase f(193–202)	1128·67	LFDKPVSPLL	gi 60097925
GA3PDH f(231–240)	1115·62	FRVPTPNVSV	gi 77404273
Myoglobin f(147–154)	920·50	YKVLGFHG	gi 73586735
Beef – jejunum			
Actin, skeletal muscle f(21–30)	976·45	AGFAGDDAPR	gi 27819614
Actin, skeletal muscle f(31–41)	1198·71	AVFPSIVGRPR	gi 27819614
Actin, skeletal muscle f(32–41)	1127·67	VFPSIVGRPR	gi 27819614
Creatine kinase f(193–201)	1015·58	LFDKPVSPL	gi 60097925
Creatine kinase f(194–201)	902·50	FDKPVSPL	gi 60097925
GA3PDH f(231–240)	1115·62	FRVPTPNVSV	gi 77404273

GA3PDH, glyceraldehyde 3-phosphate dehydrogenase.
* Peptides were identified by searching the National Center for Biotechnology Information nr protein database. Listed peptides had an alimentary origin and were found in at least three of the four animals.

transformation or absorption. For these reasons, peptide identification was limited to samples collected in the first 3 h following the meal. Based on masses of peptides present in the two types of muscle foods constituting the meals[10,11], it appears that none of those dietary oligopeptides were found in digesta. This suggests that food-borne peptides were either degraded and/or that they were present in too low quantity to be detected. The latter is supported by the complex chromatographic profiles of digesta compared with the MS results, suggesting that only a fraction of the peptides was characterised and identified. It can reasonably be assumed that, within the size range studied, the identified peptides were those most important quantitatively, which is further reinforced by the fact that all identified peptides were fragments of abundant muscle proteins. Therefore, the relative contribution of peptides brought directly as constituents of cooked fish or meat to the peptide composition in digesta is likely to be very limited.

The size characteristics and identification of reproducibly detected peptides provided several types of information on digestive events and peptide release in digesta. First, a noteworthy characteristic of the detected peptides was their relatively small size, as indicated by MALDI-ToF spectra where very few compounds at $m/z > 2000$ were found. Data on the size of peptides generated *in vivo* during digestion of proteins by gastric or intestinal enzymes are scarce. In human subjects, several peptides of six to twelve amino acids were identified in the stomach and in the duodenum after ingestion of milk or yogurt[17]. This peptide length corresponds well to sizes we observed. In an *in vitro* study focusing on bonito muscle treated by artificial gastric juice[18], all proteins were converted into peptides (< 10 kDa) after 5 min. Some peptides were identified in samples collected after 1 h of artificial digestion and, again, their lengths (eight to fifteen amino acids) were consistent with sizes we observed *in vivo*. In both studies, the analytical

Table 3. Bioactive sequences contained within peptides identified in digesta in the first 3 h following ingestion of beef or trout

Bioactive sequence	Peptide containing the sequence	Food ingested	Biological activity
PR*	AGFAGDDAPR	Trout, beef	Antihypertensive
	AGDDAPRAVF	Trout, beef	
	AVFPSIVGRPR	Trout, beef	
	VFPSIVGRPR	Beef	
	PSIVGRPR	Trout	
VF*†	AGDDAPRAVF	Trout, beef	Antihypertensive
	AVFPSIVGRPR	Trout, beef	
	VFPSIVGRPR	Trout, beef	
RP‡	AVFPSIVGRPR	Trout, beef	Antihypertensive
	VFPSIVGRPR	Trout, beef	
	PSIVGRPR	Trout	
FP*	AVFPSIVGRPR	Trout, beef	Antihypertensive
	VFPSIVGRPR	Trout, beef	
GRP*†	AVFPSIVGRPR	Trout, beef	Antihypertensive
	VFPSIVGRPR	Trout, beef	
	PSIVGRPR	Trout	
IVGRPR†	AVFPSIVGRPR	Trout, beef	Antihypertensive
	VFPSIVGRPR	Trout, beef	
	PSIVGRPR	Trout	
VAP*	LRVAPEEHPTL	Trout, beef	Antihypertensive
	LRVAPEEHPT	Trout, beef	
	RVAPEEHPTL	Trout	
YALPHA*†	YALPHAIMRL	Beef	Antihypertensive
	YALPHAIM	Trout, beef	
ALPHA*†	YALPHAIMRL	Beef	Antihypertensive
	YALPHAIM	Trout, beef	
RL*	YALPHAIMRL	Beef	Antihypertensive
	TVRLEKPASY	Trout	
YL*†	DLAGRDLTDYL	Beef	Antihypertensive, opioid
KY†	KIKIIAPPERKY	Trout	Antihypertensive
RY*†	YNLKERY	Trout	Antihypertensive
IKP*†	YFKIKPLL	Trout, beef	Antihypertensive
HL*	NSYEEALDHL	Trout	Antioxidative
VSP*	LFDKPVSPLL	Trout, beef	Antihypertensive
	FDKPVSPL	Beef	
LF*†	LFDKPVSPL	Trout, beef	Antihypertensive

* BioPep database[13].
† Vercruysse *et al.*[6].
‡ Sentandreu & Toldrá[14].

procedure involved separation of compounds by reverse-phase HPLC, which might create a bias in the size of separated and identified peptides. Nevertheless, the present results corroborate that, in the molecular weight range 0–5 kDa, mainly short peptides were released by digestive proteolytic enzymes. In other words, the first steps of protein intestinal digestion were fast and efficient enough to prevent accumulation of intermediary degradation products.

Another indication on digestive proteolytic functions resides in the number of reproducibly detected peptides. The larger number in duodenum digesta, i.e. after gastric digestion mainly performed by pepsin, results most probably from a more homogeneous digestion process. In the jejunum, where also several pancreatic enzymes intervene, the chromatographic profiles suggest that more peptides were produced, but the shorter list of reproducible peptides indicates a higher composition diversity.

Peptides identified in duodenum digesta were characteristic of pepsin hydrolysis: when combining results for trout and beef, out of eighteen different peptides, twelve possessed an N-terminal Y, F or L and nine peptides possessed a C-terminal residue M, F, or L, the most frequent amino acids after digestion by pepsin[19]. In the luminal phase of the small intestine, products of peptic degradation are further cleaved by pancreatic proteases (trypsin, α-chymotrypsin, elastase and carboxypeptidase A and B) at more alkaline pH. We confirmed in the present study that reproducibly detected peptides in the jejunum were a mixture of peptides generated upstream and withstanding proteolysis entirely (for example, FRVPTPNVSV) or partly (for example, LFDKPVSPL, most probably issued from LFDKPVSPLL), and of newly generated peptides. Excluding FRVPTPNVSV which was not affected by pancreatic enzymes, out of nine peptides, three had a C-terminal leucine which is characteristics of hydrolysis by porcine chymotrypsin C[20] and four had a C-terminal arginine which is characteristics of tryptic hydrolysis[21].

More generally, detected or identified peptides were probably among the most resistant to proteolysis. This is supported by the high proportion of proline in their sequences. Proline-containing peptides were reported to be generally resistant to degradation by digestive enzymes[22–24]. Accordingly, the proportion of proline in identified peptides was well above the average abundance in fish or meat proteins[25].

Finally, the last information drawn from characterisation of peptides in digesta after a meal of trout or beef is a certain similarity regarding peptides release (six and four

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common peptides in the duodenum and jejunum, respectively). This is all the more remarkable since the two types of foods were chosen and prepared especially to show large differences in terms of collagen content, texture, structure etc. The difference between the two types of foods concerned the quantity of peptides released, which seemed slightly higher for trout as indicated by all methods, reverse-phase HPLC, MALDI-ToF and nano-LC-ESI-IT MS/MS. Similarly to DM flux, the difference between the two types of foods may be linked to the looser structure of trout, improving accessibility of proteolytic enzymes to their targets. However, resemblance in peptide release was in line with the previously mentioned efficiency of the digestive hydrolytic systems, able to some extent to function similarly on foods of different structures.

Most of bioactive sequences found within detected peptides in digesta corresponded to antihypertensive activity. Many muscle-derived ACE inhibitory peptides have been described. For example, LKPNM has been identified as an ACE inhibitor in thermolysin-digested dried bonito[26]. Katayama *et al.*[27] isolated an ACE inhibitory peptide (RMLGQTPTK) from porcine troponin C hydrolysed with pepsin. In thermolysin digest of porcine skeletal muscle, Arihara *et al.*[7] evidenced two myosin heavy chain fragments (MNPPK and ITTNP) with ACE inhibitory activity. More recently, Jang & Lee[28] purified an ACE inhibitory peptide (VLAQYK) from the hydrolysate of sarcoplasmic protein extracts from beef rump by using the combination of proteinases A and thermolysin. None of these fragments were identified in the digesta in the present study. Nevertheless, out of twenty-six identified peptides, nineteen contained sequences with potential bioactivity. ACE inhibitory sequences may be released by peptidases associated with the brush-border membrane of the enterocytes or to intracellular peptidases[29]. Under these conditions, our findings would confirm a previous suggestion that ACE inhibitory peptides may be generated in the gastrointestinal tract after ingestion of muscle foods[7]. To exert their biological activity, the ACE inhibitory peptides contained within oligopeptides we have described also have to reach the bloodstream. The possibility of absorption into the circulatory blood system of an antihypertensive dipeptide has been demonstrated in human subjects[30]. For larger peptides, although mechanisms of absorption have not been thoroughly studied, an ultimate impact on blood pressure has been demonstrated after oral administration to spontaneously hypertensive rats. This applies, for example, to peptides derived from porcine skeletal muscle myosin[8] or to the bonito-derived peptide LKPNM[31].

In conclusion, the present study showed that after fish or meat ingestion, among the wide range of peptides produced by enzymic digestion, some of them can be reproducibly observed in intestinal digesta. Some peptides have been shown to have a local action in the intestine[2]. However, the biological activity of peptides is generally peripheral, thereby implying peptide absorption. Although absorption has been occasionally proven for particular peptides, this topic deserves further studies.

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