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Hydrolyzed proteins from herring and salmon rest raw material contain peptide motifs with angiotensin-I converting enzyme inhibitors and resulted in lower urine concentrations of protein, cystatin C and glucose when fed to obese Zucker fa/fa rats

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ABSTRACT

The use of angiotensin-I converting enzyme (ACE) inhibitors is a common strategy for treating kidney disease. Several amino acid sequences with ACE inhibiting activity are identified in filet and rest raw material from various species of fish, and fish protein hydrolysates could be of interest for possible treatment or prevention of kidney disease. Therefore, we hypothesized that protein hydrolysates from rest raw material from herring and salmon contained ACE inhibiting motifs, and could beneficially affect typical markers for kidney function in an obesity rat model prone to developing renal failure. We identified 81 and 49 peptide sequences with known ACE inhibiting activity in herring and salmon protein hydrolysates from rest raw material, respectively. To investigate the effects of fish protein hydrolysates on markers of kidney function, obese Zucker fa/fa rats consumed diets with 25% of protein from herring (HER) or salmon (SAL) protein hydrolysate from rest raw material and 75% of protein from casein/whey, or 100% protein from casein/whey (CAS) for 4 weeks. Rats fed HER or SAL diets had lower urine concentrations (relative to creatinine) of protein, cystatin C and glucose when compared to rats fed CAS diets, with no differences between groups for serum concentrations of protein, creatinine and cystatin C. To conclude, protein hydrolysates from herring and salmon rest raw material contained several peptide sequences with known ACE inhibiting activities, and resulted in lower urine concentrations of proteins, cystatin C and glucose when fed to obese Zucker rats.

Abbreviations: ACE, angiotensin-I converting enzyme; CAS, control diet containing casein; HER, diet containing herring protein hydrolysate; SAL, diet containing salmon protein hydrolysate.

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1. Introduction

Obesity is an important and independent risk factor for development of chronic renal diseases [1-4], and this is of concern as the prevalence of obesity is increasing in the world [5]. To reduce blood pressure and protect kidneys, patients with kidney disease are often treated with angiotensin-I converting enzyme (ACE) inhibitors to delay the progression of chronic kidney disease and amend proteinuria [6-8]. ACE catalyzes the conversion of angiotensin-I to the potent vasoconstrictor angiotensin-II and therefore plays an important role in the regulation of blood pressure. Peptides with ACE inhibiting capacities in vitro have been identified in various marine sources such as muscle, skin and frame from fish, microalgae, shrimp, clam and sea cucumber [9].

The obese Zucker fa/fa rat has an abnormal lipid metabolism and presents changes often seen in human obesity, and is the most used and most representative rat model for studies of metabolic complications and possible treatments of obesity [10]. Zucker fa/fa rats develop a range of abnormalities resembling human metabolic syndrome, including insulin resistance, dyslipidemia, mild glucose intolerance, hyperinsulinemia and renal damage [10]. With increasing age, these rats spontaneously develop proteinuria and focal segmental glomerulosclerosis ultimately leading to renal failure [11-13]. In addition, the Zucker fa/fa rat develops hypertension, possibly through increased renal sodium reabsorption caused by hyperinsulinemia. Obesity is associated with proteinuria and focal segmental glomerulosclerosis, and increases the risk of developing renal disease both in in humans and rats [1-4,13]. The use of obese rat models that spontaneously develop renal disease, such as the obese Zucker fa/fa rat, is highly relevant to understanding the pathophysiology of obesity associated morbidity in humans [13,14].

More knowledge about how various types of dietary proteins may affect kidney function is warranted, both for the general public and for individuals with high risk of developing impaired kidney function. We have recently reported that protein hydrolysates from rest raw materials of herring and salmon contain motifs with hypocholesterolemic or antidiabetic activities, and we demonstrated that diets containing these protein hydrolysates resulted in lower cholesterol levels and improved postprandial glucose regulation, respectively, in obese Zucker fa/fa rats [15]. A persistent high blood glucose level over time may damage the glomeruli and thus compromise the capacity of the kidneys to filter the blood. The beneficial effect of the hydrolysates, especially that of salmon protein hydrolysate on postprandial glucose regulation [15] inspired us to expand the study to also investigate effects of these fish protein hydrolysates on markers of renal function in rats. Therefore, the objective of this study was to investigate whether hydrolysates from rest raw material from herring and salmon proteins contained ACE inhibiting motifs, and if these hydrolysates would affect typical markers for kidney function in an obese rat model prone to developing renal failure. Large amounts of protein rich fish rest raw material such as heads, tails, guts, bones and other cut-offs are produced by the world's fisheries and aquaculture industries, but very little is used for human consumption [16]. We hypothesized that protein hydrolysates from herring and salmon rest raw material contained ACE inhibiting motifs, and could beneficially affect typical markers for kidney function in obese Zucker fa/fa rats which spontaneously develop renal failure. To test this hypothesis, we first examined if the hydrolyzed protein from rest raw materials from herring and salmon contained known motifs with ACE inhibiting potential, and second, we investigated if these protein hydrolysates affected markers of kidney function in serum and urine from obese Zucker fa/fa rats.

2. Methods and materials

2.1. Animals and diets

The design of this study and the diets has been described by Drotningsvik et al. [15]. In brief, eighteen male Zucker fa/fa rats (HsdOla:Zucker-Lepr, from Harlan Laboratories, The Netherlands) were assigned to three experimental groups of six rats each with comparable mean body weight, and were housed in pairs in Makrolon IV cages (EHRET GmbH & Co.). The intervention period started when the rats were 9-10 weeks old and weighing 350 ± 20g.

The diets were prepared according to the AIN-93G recommendations for growing rats [17] (Table 1) with the exception of the protein sources. In the AIN-93G diet, casein is the sole protein source. In the present study, casein was replaced with a casein/whey mixture (90% casein, 10% whey, from KAPA JP, Armor Proteines, France) in the control diet (CAS diet). In diets containing fish protein hydrolysates, we used a blend of 15 wt% proteins from casein/whey and 5 wt% proteins from herring hydrolysate (HER diet), and 15 wt% proteins from casein/whey, and 5 wt% proteins from salmon hydrolysate (SAL diet). Protein hydrolysates from fresh rest raw materials from Norwegian

Table 1 – Ingredient composition of the diets fed to rats (g/kg diet).

	CAS	HER	SAL
Casein protein [1]	222.22	170.45	170.45
Herring hydrolysate [2]		113.60	
Salmon hydrolysate [3]			113.60
Cornstarch	505.61	443.74	443.74
Sucrose	90.00	90.00	90.00
Cellulose	50.00	50.00	50.00
Soybean Oil	70.00	70.00	70.00
t-Butylhydroquinone	0.014	0.014	0.014
Mineral Mix (AIN-93-MX)	35.00	35.00	35.00
Vitamin Mix (AIN-93-VX)	10.00	10.00	10.00
L-Methionine	1.60	1.60	1.60
L-Cystine	3.00	3.00	3.00
Choline Bitartrate [4]	2.50	2.50	2.50
Growth and Maintenance Supplement (#410751) [5]	10.00	10.00	10.00

HER, diet containing herring protein hydrolysate; SAL, diet containing salmon protein hydrolysate; CAS, casein/whey diet (control diet).
[1] contains 90 % crude protein.
[2] contains 44 % crude protein, 15% maltodextrin, 36% ash, 3% moisture.
[3] contains 44 % crude protein, 15% maltodextrin, 31% ash, 5% moisture.
[4] contains 41 % choline
[5] contains vitamin B12 (40 mg/kg) and vitamin K1 (25 mg/kg) mixed with sucrose (995 g/kg) and dextrose (5 g/kg).