



Herring and beef meals lead to differences in plasma 2-aminoaidipic acid, β -alanine, 4-hydroxyproline, cetoleic acid and docosahexaenoic acid

Downloaded from: <https://research.chalmers.se>, 2025-12-05 03:12 UTC

Citation for the original published paper (version of record):

Ross, A., Svelander, C., Undeland, I. et al (2015). Herring and beef meals lead to differences in plasma 2-aminoaidipic acid, β -alanine, 4-hydroxyproline, cetoleic acid and docosahexaenoic acid concentrations in overweight men. *Journal of Nutrition*, 145(11): 2456-2463. <http://dx.doi.org/10.3945/jn.115.214262>

N.B. When citing this work, cite the original published paper.

Herring and Beef Meals Lead to Differences in Plasma 2-Aminoadipic Acid, β -Alanine, 4-Hydroxyproline, Cetoleic Acid, and Docosahexaenoic Acid Concentrations in Overweight Men^{1–3}

Alastair B Ross,^{4*} Cecilia Svelander,⁴ Ingrid Undeland,⁴ Rui Pinto,^{5,6} and Ann-Sofie Sandberg⁴

⁴Food and Nutrition Science, Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden; ⁵Bioinformatics Infrastructure for Life Sciences, Linköping, Sweden; and ⁶Computational Life Science Cluster, Department of Chemistry, Umeå University, Umeå, Sweden

Abstract

Background: Dietary guidelines generally recommend increasing fish intake and reducing red meat intake for better long-term health. Few studies have compared the metabolic differences between eating meat and fish.

Objective: The objective of this study was to determine whether there are differences in the postprandial plasma metabolic response to meals containing baked beef, baked herring, and pickled herring.

Methods: Seventeen overweight men (BMI 25–30 kg/m², 41–67 y of age) were included in a randomized crossover intervention study. Subjects ate baked herring-, pickled herring-, and baked beef-based meals in a randomized order and postprandial blood plasma samples were taken over 7 h. Plasma metabolomics were measured with the use of gas chromatography–mass spectrometry and areas under the curve for detected metabolites were compared between meals.

Results: The plasma postprandial response of 2-aminoadipic acid, a suggested marker of diabetes risk, was 1.6 times higher after the beef meal than after the baked herring meal ($P < 0.001$). Plasma β -alanine and 4-hydroxyproline both were markedly greater after beef intake than after herring intake (16 and 3.4 times the response of baked herring, respectively; $P < 0.001$). Herring intake led to a greater plasma postprandial response from docosahexaenoic acid (DHA) and cetoleic acid compared with beef (17.6 and 150 times greater, respectively; $P < 0.001$), whereas hippuric acid and benzoic acid were elevated after pickled herring compared with baked herring (5.4 and 43 times higher; $P < 0.001$).

Conclusions: These results in overweight men confirm that DHA and cetoleic acid reflect herring intake, whereas β -alanine and 4-hydroxyproline are potential biomarkers for beef intake. The greater postprandial rise in 2-aminoadipic acid after the beef meal, coupled to its proposed role in stimulating insulin secretion, may have importance in the context of red meat intake and increased diabetes risk. This trial was registered at clinicaltrials.gov as NCT02381613. *J Nutr* 2015;145:2456–63.

Keywords: metabolomics, herring, beef, plasma biomarker, diabetes, 2-aminoadipic acid, beta alanine, 4-hydroxyproline

Introduction

Current dietary guidelines advise increased intake of fish and other seafood in place of meat (1). Greater intake of fish is associated with a decreased risk of lung and colorectal cancer (2, 3) and childhood allergy (4, 5), as well as a reduction in coronary

heart disease and stroke risk (6), whereas a high intake of red meat is generally associated with negative health outcomes, including an increased risk of cardiovascular disease and cancer (6–9). In spite of the fact that red meat and fish are important dietary protein sources, relatively few intervention studies have compared meat and fish intakes. Among the findings in studies comparing meat and fish intakes, short- to medium-term fish

¹ Supported by the Swedish Board of Agriculture (formerly National Board of Fisheries; project no. 031-0267-08, European Fisheries Fund Investing in Sustainable Fisheries); the Region of Västra Götaland (project RUN 612-11-02-08); and the Swedish Research Council for Environmental Science, Agricultural Science and Spatial Planning (project no. 222-2011-1322).

² Author disclosures: AB Ross, C Svelander, I Undeland, R Pinto, and A-S Sandberg, no conflicts of interest.

³ Supplemental Figure 1 and Supplemental Tables 1–3 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

*To whom correspondence should be addressed. E-mail: Alastair.Ross@chalmers.se.

TABLE 1 Macronutrient and energy content of the 3 test meals¹

Meal	Fat, g	Protein, g	Carbohydrate, g	Energy, kJ
Baked herring	29	33	47	2416
Pickled herring	29	29	81	2912
Baked beef	35	43	47	2822

¹ Macronutrient composition data are based on analyzed values (17) for beef and fish, and from the Swedish food composition database (<http://www7.slv.se/SokNaringsinnehall>) for the composition of the accompanying potatoes and sour cream.

intake increased HDL cholesterol (10, 11), reduced blood pressure (12), and increased insulin sensitivity (13). These results imply that eating more fish is beneficial to health, yet beyond the well-researched area of ω -3 FAs and high concentrations of selenium and vitamin D, little is known about the wider metabolic effects of eating fish compared with red meat.

Rodent studies have indicated that long-term fish intake can have a wide impact on gene expression (14). Because metabolites are the end product of gene expression and reflect the interaction between environment and genotype, using global metabolite profiling (metabolomics) could be a way of better understanding the different ways that fish and meat interact with and affect human endogenous metabolism. Metabolomics is now widely used in human nutrition research (15), and fasting blood plasma samples are often used to search for effects on metabolic homeostasis. This is attractive, because it represents metabolism at a relatively steady state. However, humans are most frequently in a postprandial state, and metabolite concentrations are highly dynamic in response to the intake of food, so measuring only fasting plasma samples may mean that we are missing many of the metabolic changes occurring after a meal (16).

In order to probe the differences in metabolic response between fish and meat beyond n -3 FAs, we performed a randomized crossover study with meals based on herring (*Clupea harengus*), a commonly consumed fish in Northern Europe, and beef. Herring can be prepared in several ways, including baking and pickling. In this study, we compared the postprandial metabolic response of baked herring and pickled herring with baked ground beef in overweight male subjects with the aim of

finding metabolites that could be biomarkers of fish and meat intakes, and possibly explaining the difference in intrinsic physiologic response to these 2 sources of dietary protein.

Methods

Clinical study. All study participants gave written informed consent before participating in the study. The study design, subject characteristics, and meal composition have been described in detail elsewhere (17). Briefly, the study participants ($n = 17$, male, BMI 26.4–29.5 kg/m², age 41–67 y) consumed 3 test meals in a crossover design with a randomized meal order and with 1 wk washout between the meals. The study was approved by the regional ethical committee at the University of Uppsala and performed at Foodfiles (former KPL Good Food Practice) in accordance with the Helsinki Declaration of 1964 with later revisions. The trial was registered at clinicaltrials.gov (NCT02381613). The study meals all included 250 g boiled potatoes, 50 g sour cream (12% fat) and 2 g chives, and were served with 150 g of either baked herring fillets (125°C, 10 min, core temperature 55°C), pickled herring (industrially cured fillets in salt and acetic acid, marinated in sugar, vinegar, salt, and benzoic acid) or baked ground beef patties (140°C, 30 min, core temperature 73°C). The macronutrient composition and energy contents of the meals are given in Table 1.

The subjects were instructed to abstain from alcohol consumption and heavy exercise 36 h before the study days, and not to eat or drink anything except water after 2200 on the day before the study days. On the study days, the subjects arrived fasted at the study center at 0730 and stayed at the center until the last blood sample was taken at 1800. The subjects were served a standardized breakfast (200 g low-fat yogurt, 100 g orange juice, 2 slices of white bread, 10 g butter, and 10 g jam) at 0800 and the study meals were served at 1100. The first blood sample was taken just before the study meal (time = 0 h) and samples were then withdrawn for analysis every hour until 1800 (time = 7 h). Plasma samples were collected into EDTA-coated tubes and centrifuged within a few minutes at 1000 \times g at 6°C for 10–12 min, and then frozen at –20°C. The tubes were moved to –80°C within 2 d for long-term storage.

Plasma and food analysis. Plasma metabolomics analysis was carried out with the use of the protocol from A et al. (18). Briefly, 100 μ L plasma was mixed with 900 μ L of an ice-cold methanol:water (90:10 vol:vol) extraction mix that contained 10 internal standards. The plasma was extracted in a bead mill for 2 min at 30 Hz, allowed to settle for 2 h at

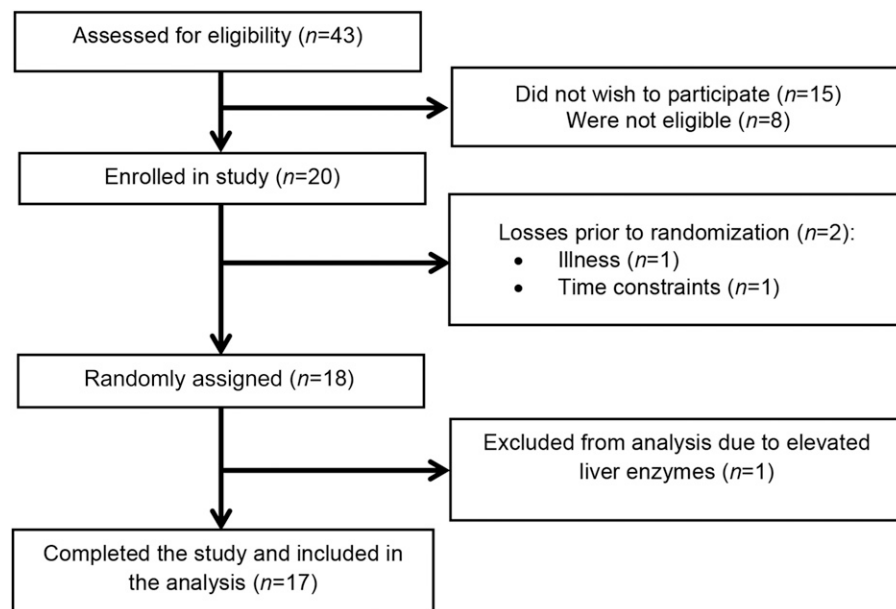
**FIGURE 1** Flow of participants through the study.

TABLE 2 Plasma metabolite AUC responses that differed after beef- or herring-based meals in overweight men¹

Metabolite	AUC, GC-MS response · h			P
	Baked herring	Baked beef	Pickled herring	
Increased with meat intake				
2-Aminoadipic acid	8 ± 1 ^a	12 ± 1 ^b	5 ± 1 ^a	<0.001
4-Hydroxyproline	314 ± 79 ^a	1060 ± 170 ^b	252 ± 75 ^a	<0.001
β-Alanine	21 ± 12 ^a	325 ± 22 ^b	13 ± 15 ^a	<0.001
Creatinine	952 ± 176 ^a	1280 ± 198 ^b	182 ± 125 ^c	0.001
Increased with herring intake				
DHA	97 ± 14 ^a	6 ± 5 ^b	90 ± 15 ^a	<0.001
FA 22:1 ²	1520 ± 193 ^a	-18 ± 19 ^b	1570 ± 262 ^a	<0.001

¹ Values are means ± SEMs divided by 1000, *n* = 17. Named metabolites are those that had a combined retention index and mass spectra matching score of over 90%. Means in a row without a common letter differ at the *P* value indicated in the same row. GC-MS, gas chromatography–mass spectrometry.

² Based on differences in FA composition of the meals (17), we tentatively identify this metabolite as cetoleic acid.

4°C, and centrifuged at 15,000 × *g* for 10 min. A total of 200 μL of the supernatant was transferred to chromatography vials and completely evaporated under vacuum. Samples were then derivatized with the use of methoxymation and *N*-methyl-*N*-trimethylsilyltrifluoroacetamide + 1% trimethylchlorosilane before injection on a Leco Pegasus III gas chromatograph coupled to a time-of-flight mass spectrometer (Leco).

The 3 protein sources fed to the subjects in the trial were analyzed with the use of a procedure similar to that used for the plasma with some slight modifications. Approximately 150 mg frozen fish or beef was weighed into a microcentrifuge tube, and 900 μL extraction mix (as above) and 200 μL chloroform were added. Metal beads were added to the tubes and the samples extracted in a bead mill for 5 min at 30 Hz. All other procedures were the same as for the plasma samples.

Data analysis. Gas chromatography–mass spectrometry data were exported from ChromaTOF software and all data processing was carried out with the use of MATLAB scripts. Peaks were aligned based on the internal standards and peak deconvolution and picking were performed with the use of 2 different scripts, “targeted” and “untargeted,” the first identifying peaks from an in-house library (Swedish Metabolomics Centre) based on matching retention index and mass spectra, and the second with the use of hierarchical multivariate curve resolution (19) to deconvolute all peaks within the chromatogram. Peak areas were normalized to the internal standard responses (α-ketoglutarate, glutamic acid, myristic acid, salicylic acid, and succinic acid) with the use of principal component analysis (PCA) before statistical analyses (17). Initial data analysis by PCA found 3 clear outliers, which were the result of bad chromatography (poor overall peak shape, including internal standards). Data from these samples were removed from the dataset and replaced by the mean of the 2 adjacent time points during further data analysis.

Food data were handled in the same manner as plasma samples, except that food data were further corrected for sample weight.

Data from both the targeted and untargeted peak picking were analyzed with the use of a mixed model to determine differences in overall plasma response between 0 and 7 h. Meal type and time were included as fixed factors, with meal type modeled for unequal variances. Data were manually corrected for baseline ($T_{1...7h} - T_0$) before analysis. Peaks from the untargeted analysis in which diet-related differences were identified (*P* < 0.05) were further investigated for identification with the use of internal and National Institute of Standards and Technology mass spectral libraries.

Differences from diet at 7 h postmeal were tested with the use of the General Linear Model, with subject included as a covariate. Tukey's post hoc test was used to determine differences between groups. Data were not corrected for baseline values in order to simulate a nonrepeated

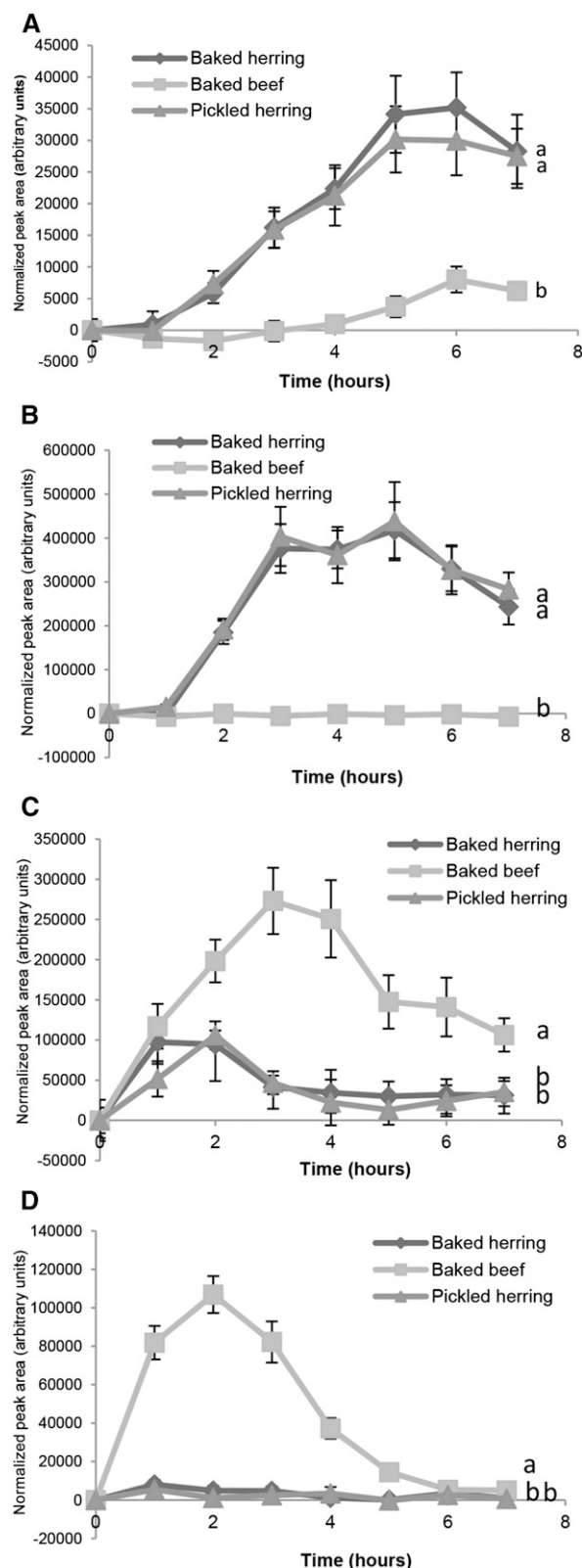


FIGURE 2 Plasma metabolite responses for DHA (A), FA 22:1 (B), 4-hydroxyproline (C), and β-alanine (D) after single meals based on baked herring, baked beef, or pickled herring in overweight men. Values are means ± SEMs of a normalized gas chromatograph–mass spectrometer response, *n* = 17. Different lower case letters for plasma curves indicate overall differences in the plasma AUC between the meals (*P* < 0.05).

TABLE 3 Metabolites that differed in plasma response over 7 h after a baked beef– compared with a baked herring–based meal in overweight men¹

Metabolite	AUC, GC-MS response · h		P
	Baked herring	Baked beef	
Increased after intake of baked herring compared with baked beef			
Rhamnose	124 ± 49	−1 ± 45	0.003
Increased after intake of baked beef compared with baked herring			
2-Aminoadipic acid	8 ± 1	12 ± 1	<0.001
5-Methylcysteine	−2320 ± 1680	1190 ± 2160	0.01
3-Oxoglutaric acid	−165 ± 336	551 ± 363	0.03
Isomaltose	−36 ± 90	152 ± 50	0.004
Lactulose	−284 ± 292	255 ± 147	0.004
Leucine	6170 ± 665	7470 ± 4220	0.06
Norleucine	4560 ± 309	5330 ± 415	0.05
N-acetylmannosamine	−1490 ± 701	414 ± 964	0.007

¹ Values are means ± SEMs divided by 1000, *n* = 17. Named metabolites are those that had a combined retention index and mass spectra matching score of over 90%. The plasma response of all metabolites in the table differs for the 2 diets at the *P* value indicated in the same row. GC-MS, gas chromatography–mass spectrometry.

measures situation that would likely be encountered when using biomarkers to determine dietary intake.

NCSS for Windows 9.0.5 was used for all mixed-model and univariate data analyses. Uncorrected *P* values < 0.05 are reported as of interest in line with the exploratory nature of this work. Biologically meaningful differences of *P* < 0.1 are also reported.

Differences in plasma response between the meals were investigated with PCA and orthogonal partial least squares discriminant analysis (OPLS-DA) (20) with the use of SIMCA software (Umetrics). After normalization based on internal standard response, data were centered per person by subtracting the average area of all time points for one subject from each time point for that subject, and data were subjected to unit variance scaling and mean centering. The plasma responses to the baked herring meal were compared with the pickled herring and baked ground beef, respectively, in 2 separate OPLS-DA analyses, and in both cases the difference between the meals was explained by the first predictive component. The OPLS-DA models were made from all time points in the dataset, i.e., 0–7 h, as well as for only time points 2 and 3 h, or 6 and 7 h. Crossvalidation was performed by leaving out all samples from one subject for each crossvalidation test. Crossvalidation was used to estimate model complexity (number of OPLS-DA loadings) and loadings CIs through jackknifing. Model validation was based on crossvalidation scores and permutations tests with 500 permutations and the same number of components as the models to be tested.

Discriminating variables detected with the use of OPLS-DA were further analyzed for differences between diets with the use of *t* tests, with Benjamini-Hochberg–corrected *P* values used to determine significance.

Foodomics data analysis. As single samples of each food were analyzed, a factor of ±25% was added to each value to account for possible variation based on both differences in the raw materials and analytical variation. Data after normalization based on sample weight and internal standard response was then analyzed with the use of OPLS-DA to find those variables that varied the most between the different protein sources.

Selection of metabolites of interest. The results from the multivariate data analysis by OPLS-DA, the mixed-model analysis, and the *t* test were compared, and metabolites that were significant in at least one of the OPLS-DA models and one of the mixed-model or *t* tests were considered to be likely discriminatory biomarkers related to one or more of the diets.

Results

Clinical trial. Plasma TG, total, LDL, and HDL cholesterol, and serum insulin results have been previously reported for this study (17). Of the 20 subjects that started the study, 17 completed it,

TABLE 4 Metabolites that differed in plasma response over 7 h after a baked herring– compared with a pickled herring–based meal in overweight men¹

Metabolite	AUC, GC-MS response · h		P
	Baked herring	Pickled herring	
Increased after intake of baked herring compared with pickled herring			
Aspartic acid	27 ± 2	17 ± 3	0.01
Creatinine	952 ± 176	182 ± 125	0.002
Leucine	6170 ± 665	4220 ± 555	0.007
Norleucine	4560 ± 309	3150 ± 243	0.003
Increased after intake of pickled herring compared with baked herring			
Benzoic acid	−80 ± 40	43 ± 25	0.001
Hippuric acid	63 ± 39	338 ± 57	<0.001
Isomaltose	−36 ± 90	202 ± 164	0.02
N-acetylmannosamine	−1490 ± 701	111 ± 716	0.009

¹ Values are means ± SEMs divided by 1000, *n* = 17. Named metabolites are those that had a combined retention index and mass spectra matching score of over 90%. The plasma response of all metabolites in the table differs for the 2 diets at the *P* value indicated in the same row. GC-MS, gas chromatography–mass spectrometry.

with 2 dropping out because of prior commitments and 1 excluded on the basis of elevated liver enzyme values (alanine transaminase and aspartate transaminase) at the last time point (Figure 1). No differences were observed for postprandial lipoproteins, although TG clearance was slower after the beef meal. Both DHA and EPA were elevated after the 2 herring meals compared with the beef meal, whereas insulin response was higher after pickled herring than after both baked herring and beef because of its high sucrose content (22%) (17).

Metabolic profiling of plasma samples. In total, 50 unique metabolites responded differently to meals with baked beef, baked herring, and pickled herring intake, matched on a weight basis. Five compounds had a higher response after beef intake than after either herring meal, whereas 3 compounds were higher for the 2 herring meals than for the beef meal (Table 2, Figure 1, Figure 2, and Supplemental Table 1). When comparing only baked herring with baked beef, 8 compounds were higher after baked herring and 16 compounds were higher after baked beef (Table 3 and Supplemental Table 2). Comparing baked herring and pickled herring, 8 compounds had a higher response after eating baked herring, whereas 22 compounds were higher after pickled herring (Table 4 and Supplemental Table 3), indicating that it is possible to discriminate both origin of protein (beef compared with herring) and type of processing (baking compared with pickling). Of the 50 compounds that discriminated between baked beef and herring, or baked herring and pickled herring, we were able to identify 25 based on the fact that mass spectra and retention time matched with library compounds, and tentative compound class could be assigned to a further 6 metabolites based on mass spectra only.

There were 3 notable differences between the 2 herring meals and the beef meal: 1) beef led to a higher 2-amino adipic acid ($P < 0.001$) response and tended to lead to a higher leucine response ($P = 0.06$) (Figure 3 and Tables 2 and 3), 2) the postprandial response of the FAs DHA and cetoleic acid were higher after the 2 herring meals, and 3) the postprandial response of β -alanine and 4-hydroxyproline after the beef meal was remarkably higher than after the herring meals (Figure 2 and Table 2). Plasma creatinine response was the only identified compound that differed in plasma response between all 3 meals. When the 2 herring meals were compared, the plasma response of creatinine, leucine, and norleucine were greater after baked herring and the plasma response of 1,5-anhydro-*D*-glucitol, benzoic acid, and hippuric acid were greater after pickled herring (Table 4 and Figure 3).

In order to identify metabolites that differed after the meals under close-to-fasting conditions, and thus might warrant further investigation as biomarkers of intake in fasting blood samples, we analyzed metabolic differences in samples taken 7 h postmeal. Only 3 metabolites still differed because of the type of protein source at 7 h postmeal, with 4-hydroxyproline higher after the beef meal than after the herring meals, and DHA and FA 22:1 after the 2 herring meals than after the beef meal (Table 5).

Food metabolomics. In order to understand whether plasma metabolite differences likely were due to differences in the concentration of those metabolites in the meals, or due to an endogenous metabolic response to the meals, we analyzed the composition of the different dietary protein sources in the study with the use of metabolomics. Of the 136 metabolites found in at least one of the food samples, 52 differed between baked beef and baked herring, whereas 73 differed between baked herring and pickled herring, based on a threshold of 25% variation used

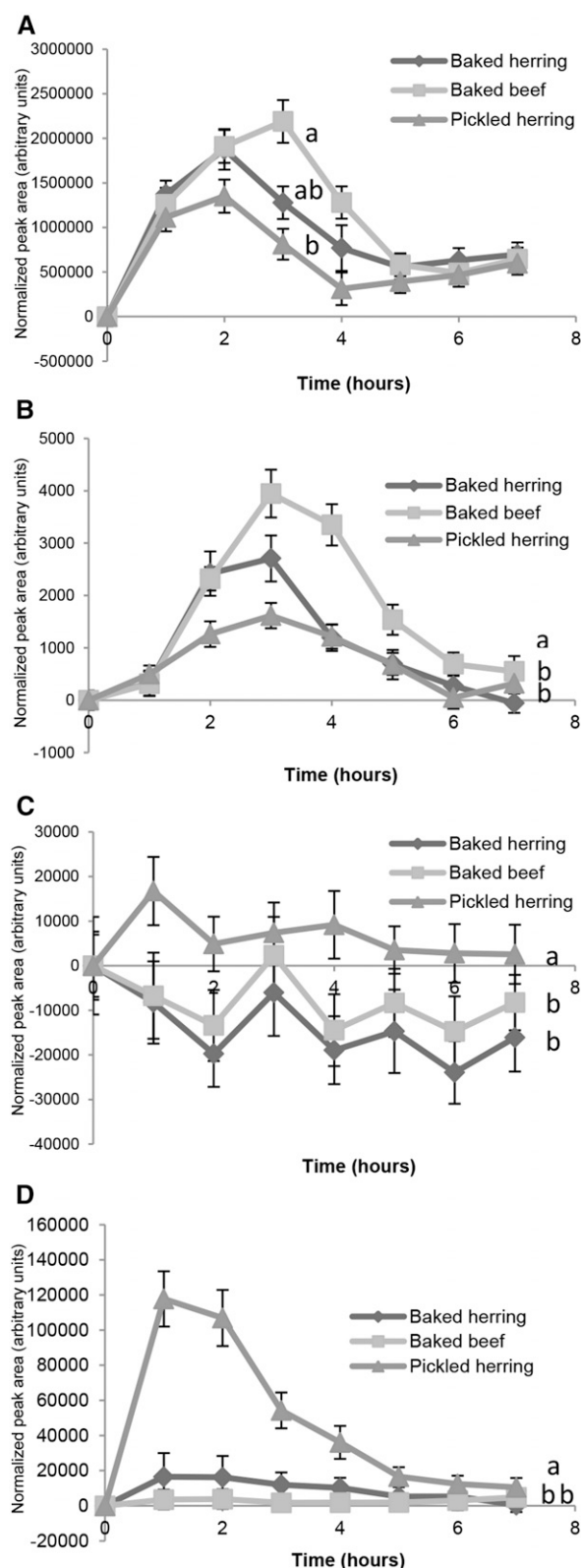


FIGURE 3 Plasma metabolite responses for leucine (A), 2-amino adipic acid (B), benzoic acid (C), and hippuric acid (D) after single meals based on baked herring, baked beef, or pickled herring in overweight men. Values are means \pm SEMs of a normalized gas chromatograph-mass spectrometer response, $n = 17$. Different lower case letters for plasma curves indicate overall differences in the plasma AUC between the meals ($P < 0.05$).

TABLE 5 Metabolites with differences at 7 h after consumption of a meal containing baked beef, baked herring, or pickled herring in overweight men¹

Metabolite	GC-MS peak area			P
	Baked herring	Baked beef	Pickled herring	
Higher after consumption of baked beef compared with the 2 herring meals				
4-hydroxyproline	254 ± 18 ^a	384 ± 21 ^b	290 ± 23 ^a	0.006
Higher after consumption of baked and pickled herring compared with baked beef				
DHA	40 ± 6 ^a	18 ± 1 ^b	38 ± 4 ^a	0.004
FA 22:1 ²	251 ± 40 ^a	3 ± 1 ^b	290 ± 38 ^a	<0.001

¹ Values are means ± SEMs divided by 1000, *n* = 17. Named metabolites are those that had a combined retention index and mass spectra matching score of over 90%. Means in a row without a common letter differ at the *P* value indicated in the same row. GC-MS, gas chromatography–mass spectrometry.

² Based on differences in FA composition of the meals (17), we tentatively identify this metabolite as cetoleic acid.

to account for potential variation because of raw ingredients, preparation, and analysis of samples derived from a single source. Among those plasma metabolites found to differ in postprandial response after the meals, 12 could be found in food, and compositional differences in food were found to match plasma response in the cases of β -alanine, benzoic acid, creatinine, DHA, and leucine (**Supplemental Figure 1**). FA analysis also confirmed that the plasma differences in DHA and cetoleic acid were due to compositional differences between beef and herring (17).

Discussion

To our knowledge, this is the first time that metabolomics has been applied to compare the different effects of meat and fish on the plasma metabolome. Several differences appear to be a simple effect of the fact that compositional differences in beef, herring, or pickled herring are reflected in the postabsorptive plasma metabolome, although others reflect endogenous responses that may be linked to the long-term physiologic effects of eating diets high in beef or herring.

Key among these was the finding that beef consumption led to a higher 2-aminoadipic acid response than did the 2 herring meals. 2-Aminoadipic acid, a metabolite in the lysine degradation pathway, has been suggested as an early biomarker for the development of diabetes in humans, and has been found to stimulate insulin secretion *in vitro* (21). Diabetes risk predicted by 2-aminoadipic acid was suggested to predict a pathway for stimulating insulin secretion unrelated to other known insulin stimulators (21). The hypothesis that 2-aminoadipic acid stimulates postprandial insulin response is not supported by our earlier findings, given that we found no difference in insulin response between baked beef and herring (17), although this study was not powered or designed to investigate differences in glucose metabolism. Another explanation for the difference is that 2-aminoadipic acid response in plasma is due to the breakdown of collagen after the beef meal, because it is also a breakdown product of collagen (22). Collagen was not measured in the different meals, but previous compositional data has found similar proportions of collagen between beef and fish, although the amino acid composition differs (23). In this study, the overall amount of protein eaten in the beef meal was higher than that for herring, meaning a likely higher intake of collagen, which also may explain the greater postprandial rise in 2-aminoadipic acid.

Preceding the rise in 2-aminoadipic acid, there was a tendency for an increase in the plasma response of the BCAA leucine after the baked beef meal compared with the baked herring meal. Because leucine and other BCAAs have been implicated in the early development of diabetes (24), it is tempting to suggest that there may be a link between the increase in plasma leucine and an increase in 2-aminoadipic acid. Earlier work has not found any association between leucine, other BCAAs, and 2-aminoadipic acid (21), although those data were based on fasting plasma samples. The metabolite profiling of the baked beef and herring did not find any 2-aminoadipic acid in either of the meals, nor any difference in its upstream metabolite lysine, nor was any difference in leucine found between the baked beef and herring meals. Differences in metabolite concentrations could be due to differential bioavailability, although we were unable to find any evidence that amino acid bioavailability was different between marine and terrestrial protein sources.

The present data are limited with respect to drawing conclusions about any long-term relation between BCAA, 2-aminoadipic acid, and insulin sensitivity, because they are based on a single meal. However, red meat intake previously has been associated with a greater risk of type 2 diabetes (25, 26), and a murine study found that eating herring as the main protein source improved long-term insulin sensitivity compared with beef (27). Fish protein also may improve insulin sensitivity compared with other animal proteins (28) in humans. The transiently greater postprandial plasma concentrations of leucine and 2-aminoadipic acid may provide a mechanistic explanation for these findings, although trials designed specifically to study glucose metabolism in response to meat and fish intakes are needed to draw firm conclusions.

Of the metabolites that increased with herring intake but not beef intake, one, DHA, is a well-established marker of fatty fish and fish oil intakes (29–31). Cetoleic acid makes up around 12% of all FAs in herring (32). Although several studies have analyzed the FA content of plasma in herring-rich diets, none have reported increases in plasma 22:1 in fasting plasma samples, possibly because 22:1 appears to be metabolized rapidly in mammals (33), disappears quickly from human breast milk (34), and is preferentially stored in adipose tissue compared with DHA and EPA (35, 36). Although unlikely to be present in fasting samples, cetoleic acid could be considered to be a selective marker for herring intake in postprandial blood samples.

The elevated response of β -alanine after the beef meal compared with the herring meals likely is due to the very high amount in the beef meal found in the metabolomic analysis

of the different protein sources studied. Similar to β -alanine, the plasma response of 4-hydroxyproline was much higher for beef than for herring. However, in this case, the amount of free-4-hydroxyproline in the baked beef and herring meals was not markedly different. Pickled herring was low in free-hydroxyproline compared with both baked beef and herring, although the plasma response of 4-hydroxyproline to both herring meals was similar. Plasma 4-hydroxyproline concentrations were still different between the beef and fish meals at 7 h postmeal, suggesting that 4-hydroxyproline could have the potential to be a marker of beef intake in fasting plasma samples. Plasma 4-hydroxyproline is also proposed as a marker of collagen turnover in tissue, being elevated in elderly and bedridden subjects (37), so high intake of beef should be considered to be a confounding factor when using 4-hydroxyproline as a marker of collagen turnover. Although several potential biomarkers for meat and fish intakes have been found in urine (38), there is a lack of validated plasma biomarkers for meat and fish intake beyond n-3 FAs for fish.

The secondary aim of this work was to find out whether a common method of processing herring—pickling—had any differential effect on metabolic response compared with baked herring. The pickling process includes the soaking of herring fillets in brine, vinegar, sugar, and benzoic acid, so it is unsurprising that benzoic acid appears in plasma after the intake of pickled herring, but not after eating baked herring. This is also the likely explanation for the high hippuric acid response. Hippuric acid is commonly found in nutritional metabolomics studies and is ascribed to various sources, including microbial fermentation of phenolic compounds and breakdown of aromatic amino acids (39). The rapid rise in hippuric acid likely is due to conjugation of benzoic acid and glycine for the rapid removal of benzoic acid from circulation.

In this work, we were able to identify several metabolites that differed in their postprandial plasma response between the different diets, but which would have been unlikely to differ had we only studied fasting plasma samples. This strength of measuring metabolomics in postprandial samples has been proposed before (16), but not regularly reported in the nutritional metabolomics literature. Similarly, metabolic profiling of food samples as a complement to plasma profiling helped to explain whether differences observed in plasma likely were due to the composition of the food or an interaction between the food and endogenous metabolism. The approach used was simple, and could be improved in future work by predigesting food samples in a gastrointestinal model (40) to better reflect the food metabolome after digestion and what would be available for uptake in the intestine.

In conclusion, these data suggest that 4-hydroxyproline and β -alanine may serve as biomarkers of beef intake, and confirm that DHA is a biomarker of fatty fish intake, likely along with cetoleic acid in nonfasting plasma samples. Further studies are needed to confirm whether 4-hydroxyproline and β -alanine could act as biomarkers of beef intake, given the lack of plasma biomarkers available. Our results also suggest that eating beef rather than fish may increase concentrations of 2-aminoadipic acid and leucine, both stimulators of insulin secretion. Although we were not able to confirm this effect based on postprandial insulin concentrations (17), the previous associations between red meat intake and diabetes warrant that the effects of chronic beef intake on nonglucose-stimulated insulin secretion be further investigated.

Acknowledgments

We thank Inga-Britt Carlsson for assistance with sample analysis, Jonas Gullberg and Thomas Moritz for help with

and access to the Swedish Metabolomics Centre, and Hans Stenlund for providing and updating the Matlab scripts used in this study. A-SS planned and initiated the study; IU and A-SS designed the study and meals; ABR and CS conducted the laboratory work; ABR, CS, and RP contributed to the data analysis; and ABR wrote the manuscript. All authors read and approved the final manuscript.

References

1. United States Department of Agriculture. Dietary guidelines for Americans 2010 [Internet]. [cited 2014 Oct 22.] Available from: <http://health.gov/dietaryguidelines/dga2010/dietaryguidelines2010.pdf>.
2. Song J, Su H, Wang BL, Zhou YY, Guo LL. Fish consumption and lung cancer risk: systematic review and meta-analysis. *Nutr Cancer* 2014;66:539–49.
3. Norat T, Bingham S, Ferrari P, Slimani N, Jenab M, Mazuir M, Overvad K, Olsen A, Tjønneland A, Clavel F, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition. *J Natl Cancer Inst* 2005;97:906–16.
4. Yang H, Xun P, He K. Fish and fish oil intake in relation to risk of asthma: a systematic review and meta-analysis. *PLoS One* 2013;8:e80048.
5. Hesselmar B, Saalman R, Rudin A, Adlerberth I, Wold A. Early fish introduction is associated with less eczema, but not sensitization, in infants. *Acta paediatr* 2010;99:1861–7.
6. Chan DS, Lau R, Aune D, Vieira R, Greenwood DC, Kampman E, Norat T. Red and processed meat and colorectal cancer incidence: meta-analysis of prospective studies. *PLoS One* 2011;6:e20456.
7. Bastide NM, Pierre FHF, Corpet DE. Heme iron from meat and risk of colorectal cancer: A meta-analysis and a review of the mechanisms involved. *Cancer Prev Res (Phila)* 2011;4:177–84.
8. Pan A, Sun Q, Bernstein AM, Schulze MB, Manson JE, Stampfer MJ, Willett WC, Hu FB. Red meat consumption and mortality: Results from 2 prospective cohort studies. *Arch Intern Med* 2012;172:555–63.
9. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013;19:576–85.
10. Lindqvist H, Langkilde AM, Undeland I, Radendal T, Sandberg AS. Herring (*Clupea harengus*) supplemented diet influences risk factors for CVD in overweight subjects. *Eur J Clin Nutr* 2007;61:1106–13.
11. Lindqvist HM, Langkilde AM, Undeland I, Sandberg AS. Herring (*Clupea harengus*) intake influences lipoproteins but not inflammatory and oxidation markers in overweight men. *Br J Nutr* 2009;101:383–90.
12. Erkkilä AT, Schwab US, de Mello VD, Lappalainen T, Mussalo H, Lehto S, Kemi V, Lamberg-Allardt C, Uusitupa MI. Effects of fatty and lean fish intake on blood pressure in subjects with coronary heart disease using multiple medications. *Eur J Nutr* 2008;47:319–28.
13. Navas-Carretero S, Perez-Granados AM, Schoppen S, Vaquero MP. An oily fish diet increases insulin sensitivity compared to a red meat diet in young iron-deficient women. *Br J Nutr* 2009;102:546–53.
14. Nookaew I, Gabrielsson BG, Holmang A, Sandberg AS, Nielsen J. Identifying molecular effects of diet through systems biology: influence of herring diet on sterol metabolism and protein turnover in mice. *PLoS One* 2010;5:e12361.
15. Jones DP, Park Y, Ziegler TR. Nutritional metabolomics: Progress in addressing complexity in diet and health. *Ann Rev Nutr* 2012;32:183–202.
16. van Ommen B, Keijer J, Heil SG, Kaput J. Challenging homeostasis to define biomarkers for nutrition related health. *Mol Nutr Food Res* 2009;53:795–804.
17. Svelander C, Gabrielsson BG, Almgren A, Gottfries J, Olsson J, Undeland I, Sandberg AS. Postprandial lipid and insulin responses among healthy, overweight men to mixed meals served with baked herring, pickled herring or baked, minced beef. *Eur J Nutr* 2015;54:945–58.
18. A J, Trygg J, Gullberg J, Johansson AI, Jonsson P, Antti H, Marklund SL, Moritz T. Extraction and GC/MS analysis of the human blood plasma metabolome. *Anal Chem* 2005;77:8086–94.
19. Jonsson P, Johansson AI, Gullberg J, Trygg JA, Grung B, Marklund S, Sjöström M, Antti H, Moritz T. High-throughput data analysis for detecting and identifying differences between samples in GC/MS-based metabolomic analyses. *Anal Chem* 2005;77:5635–42.

20. Bylesjö M, Rantalainen M, Cloarec O, Nicholson JK, Holmes E, Trygg J. OPLS discriminant analysis: combining the strengths of PLS-DA and SIMCA classification. *J Chem* 2006;20:341–51.
21. Wang TJ, Ngo D, Psychogios N, Dejam A, Larson MG, Vasan RS, Ghorbani A, O'Sullivan J, Cheng S, Rhee EP, et al. 2-Aminoadipic acid is a biomarker for diabetes risk. *J Clin Invest* 2013;123:4309–17.
22. Sell DR, Strauch CM, Shen W, Monnier VM. Aging, diabetes, and renal failure catalyze the oxidation of lysyl residues to 2-aminoadipic acid in human skin collagen: evidence for metal-catalyzed oxidation mediated by alpha-dicarbonyls. *Ann N Y Acad Sci* 2008;1126:205–9.
23. Yamada S, Yamamoto K, Ikeda T, Yanagiguchi K, Hayashi Y. Potency of fish collagen as a scaffold for regenerative medicine. *BioMed Res Int* 2014;2014:302932.
24. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab* 2012;15:606–14.
25. Schulze MB, Manson JE, Willett WC, Hu FB. Processed meat intake and incidence of Type 2 diabetes in younger and middle-aged women. *Diabetologia* 2003;46:1465–73.
26. Aune D, Ursin G, Veierod MB. Meat consumption and the risk of type 2 diabetes: a systematic review and meta-analysis of cohort studies. *Diabetologia* 2009;52:2277–87.
27. Hussain A, Nookaew I, Khoomrung S, Andersson L, Larsson I, Hulthen L, Jansson N, Jakubowicz R, Nilsson S, Sandberg AS, et al. A maternal diet of fatty fish reduces body fat of offspring compared with a maternal diet of beef and a post-weaning diet of fish improves insulin sensitivity and lipid profile in adult C57BL/6 male mice. *Acta Physiol (Oxf)* 2013;209:220–34.
28. Ouellet V, Marois J, Weisnagel SJ, Jacques H. Dietary cod protein improves insulin sensitivity in insulin-resistant men and women: a randomized controlled trial. *Diabetes Care* 2007;30:2816–21.
29. Brantsaeter AL, Haugen M, Thomassen Y, Ellingsen DG, Ydersbond TA, Hagve TA, Alexander J, Meltzer HM. Exploration of biomarkers for total fish intake in pregnant Norwegian women. *Public Health Nutr* 2010;13:54–62.
30. Chung H, Nettleton JA, Lemaitre RN, Barr RG, Tsai MY, Tracy RP, Siscovick DS. Frequency and type of seafood consumed influence plasma (n-3) fatty acid concentrations. *J Nutr* 2008;138:2422–7.
31. Vandevijvere S, Geelen A, Gonzalez-Gross M, van't Veer P, Dallongeville J, Mouratidou T, Dekkers A, Børnhorst C, Breidenassel C, Crispim SP, et al. Evaluation of food and nutrient intake assessment using concentration biomarkers in European adolescents from the Healthy Lifestyle in Europe by Nutrition in Adolescence study. *Br J Nutr* 2013;109:736–47.
32. Aro TL, Larmo PS, Bäckman CH, Kallio HP, Tahvonen RL. Fatty acids and fat-soluble vitamins in salted herring (*Clupea harengus*) products. *J Agric Food Chem* 2005;53:1482–8.
33. Cooper MH, Iverson SJ, Rouvinen-Watt K. Metabolism of dietary cetoleic acid (22:1n-11) in mink (*Mustela vison*) and gray seals (*Halichoerus grypus*) studied using radiolabeled fatty acids. *Physiol Biochem Zool* 2006;79:820–9.
34. Francois CA, Connor SL, Wander RC, Connor WE. Acute effects of dietary fatty acids on the fatty acids of human milk. *Am J Clin Nutr* 1998;67:301–8.
35. Lindqvist H, Sandberg AS, Undeland I, Stener-Victorin E, Larsson BM, Sannaveerappa T, Lonn M, Holmang A. Influence of herring (*Clupea harengus*) and herring fractions on metabolic status in rats fed a high energy diet. *Acta Physiol (Oxf)* 2009;196:303–14.
36. Lin DS, Conner WE. Are the n-3 fatty acids from dietary fish oil deposited in the triglyceride stores of adipose tissue? *Am J Clin Nutr* 1990;51:535–9.
37. Koike K, Li Y, Seo M, Sakurada I, Tezuka K, Uchikura K. Free 4-hydroxyproline content in serum of bedridden aged people is elevated due to fracture. *Biol Pharm Bull* 2000;23:101–3.
38. O'Gorman A, Gibbons H, Brennan L. Metabolomics in the identification of biomarkers of dietary intake. *Comput Struct Biotechnol J* 2013;4:e201301004.
39. Lees HJ, Swann JR, Wilson ID, Nicholson JK, Holmes E. Hippurate: The natural history of a mammalian-microbial cometabolite. *J Proteome Res* 2013;12:1527–46.
40. Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, Carriere F, Boutrou R, Corredig M, Dupont D, et al. A standardised static in vitro digestion method suitable for food - an international consensus. *Food Funct* 2014;5:1113–24.