Appetite 130 (2018) 199–208

Contents lists available at ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Meals based on cod or veal in combination with high or low glycemic index carbohydrates did not affect diet-induced thermogenesis, appetite sensations, or subsequent energy intake differently

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ARTICLE INFO

Keywords:
Fish
Protein source
Meat
Visual analog scales
Ghrelin
Weight management

ABSTRACT

The objective of this study was to investigate the acute effects of meals containing protein from either cod or veal in combination with high or low glycemic index (GI) carbohydrates, on diet-induced thermogenesis (DIT) (primary endpoint), appetite, energy intake (EI), as well as postpranidal ghrelin, glucose, and insulin responses. Twenty-three overweight men and women (mean ± SD age: 30.0 ± 7.6 y, BMI: 27.2 ± 1.4 kg/m^2) consumed 4 test meals: cod with mashed potatoes (high GI carbohydrate), cod with wholegrain pasta (low GI carbohydrate), veal with mashed potatoes, and veal with wholegrain pasta (~2010 kJ, ~25.5 E% protein, ~41.0 E% carbohydrate, ~33.5 E% fat). Energy expenditure was measured at baseline and six times postprandially, each lasting 25 min. Additionally, appetite sensations were measured every half hour. Arterialized venous blood samples were drawn every 20 min until an *ad libitum* buffet-style lunch was served 3.5 h later. DIT did not differ between test meals ($P > 0.05$), and there were no differences in appetite sensations or *ad libitum* EI (all, $P > 0.05$). Meal-time interactions were found for glucose and insulin ($P = 0.04$ and $P < 0.001$). Pairwise comparisons revealed that glucose and insulin peaks were higher after the meals with high GI carbohydrates. No differences were found between meals with cod or veal in combination with carbohydrates with low or high GI on DIT, appetite sensations, or EI in overweight men and women. However, as expected meals with high GI carbohydrates resulted in higher glucose and insulin responses compared to meals with low GI carbohydrates regardless of protein source.

1. Introduction

High-protein diets have gained in popularity as a means for both body weight loss and maintenance (Leidy et al., 2015). It is well known that meals higher in protein stimulate satiety and increase diet-induced thermogenesis (DIT) compared to meals with lower protein content (Belza et al., 2013; Halton & Hu, 2004; Karst, Steiniger, Noack, & Steglich, 1984). However, it is less well investigated whether proteins from different sources differ in their effects on appetite and DIT. In 1992, Uhe and colleagues reported increased satiety sensations and increased plasma taurine and methionine concentrations after consumption of fish (*mustelus antarcticus*) compared to beef and chicken

Abbreviations: DIT, diet-induced thermogenesis; GI, glycemic index; EI, energy intake; BMI, body mass index; VAS, visual analog scale; eVAS, electronic tablet-based visual analog scaling device; RMR, resting metabolic rate; PFC, prospective food consumption; CM, cod and mashed potatoes; CP, cod and pasta; VM, veal and mashed potatoes; VP, veal and pasta; EE, energy expenditure; iAUC, incremental area under the curve; iAOC, incremental area over the curve

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https://doi.org/10.1016/j.appet.2018.08.006
Received 13 February 2018; Received in revised form 6 August 2018; Accepted 6 August 2018
Available online 08 August 2018

(Uhe, Collier, & O'Dea, 1992). Holt et al. calculated a satiety index for 38 different foods, including six protein-rich foods (cheese, eggs, lentils, baked beans, beef steak, and ling fish) (Holt, Miller, Petocz, & Farmakalidis, 1995). They found ling fish to elicit a higher satiety index score compared to the other protein-rich foods. Pal and Ellis investigated whey, tuna, egg, and turkey, served as liquid macronutrient-balanced test meals, and found whey to be superior in stimulating satiety compared to the three other protein sources (Pal & Ellis, 2010). However, tuna increased satiety compared to egg and turkey. Borzoei et al. compared macronutrient-balanced meals with cod or beef and found a lower energy intake (EI) 4 h after the meal with cod compared to the meal with beef (Borzoei, Neovius, Barkeling, Teixeira-Pinto, & Rössner, 2006). They did not, however, observe any differences in appetite sensations between the meals. These studies indicate that protein from fish species, which generally has a higher content of methionine and taurine and a lower content of histidine compared with different meat sources ("Frida Food Data, Release 3., 2017; Gilbert, Bendsen, Tremblay, & Astrup, 2011), has some beneficial effects on appetite compared to other animal proteins. However, there are methodical shortcomings in the majority of the studies (Holt et al., 1995; Pal & Ellis, 2010; Uhe, Collier, & O'Dea, 1992). Uhe et al. did not report the total weight of the meals (Uhe et al., 1992), Pal and Ellis used liquid test meals (Pal & Ellis, 2010), and the protein content of the food items tested in the study by Holt et al. varied, with fish having the highest protein content (Holt et al., 1995). Moreover, none of the studies investigated the effect of protein source on DIT, which contributes to the beneficial effect of high-protein diets on weight loss and has been proposed as a mechanism for protein-induced satiety (Westerterp-Plantenga, Rolland, Wilson, & Westerterp, 1999). Using mice as an experimental model, a diet with cod or lean seafood has been shown to induce less body weight and fat mass gain compared to meat diets (Holm et al., 2016; Liisberg et al., 2016). It is noteworthy that the energy intake was lower when the mice were fed lean seafood compared with meat; this was accompanied by a reduced feed efficiency, indicating higher energy expenditure. Taken together, these studies indicate that lean seafood is less obesogenic than lean meat from terrestrial sources (Holm et al., 2016; Liisberg et al., 2016).

The glycemic index (GI) is a ranking of carbohydrates according to their effect on blood glucose responses (Jenkins et al., 1981). In a large European study, an *ad libitum* diet with a combination of modestly higher protein and lower glycemic index (GI) resulted in more effective weight maintenance after weight loss when compared with the official dietary guidelines (Larsen et al., 2010). Low GI meals have been demonstrated to increase satiety and reduce EI compared with high GI meals (Bornet, Jardy-Gennetier, Jacquet, & Stowell, 2007; Raben, 2002; Sun, Li, Zhang, Wong, & Wang, 2016). However, this has not been shown consistently, and the results in several of the studies may have been affected by a different fiber content in the test meals (Bornet et al., 2007; Raben, 2002; Sun et al., 2016). In mice, increased oxygen consumption and carbon dioxide production have been observed in the fed state when mice were fed a diet with fish oil in combination with low GI carbohydrates compared to a diet with fish oil in combination with high GI carbohydrates (Hao et al., 2012). These results indicate that the type of carbohydrate in the meal could influence the thermic response. The effects of high and low GI carbohydrates on DIT have been investigated in humans (Díaz, Galgani, Aguirre, Atwater, & Burrows, 2005; Kaur, Quek Yu Chin, Camps, & Henry, 2016; Keogh, Lau, Noakes, Bowen, & Clifton, 2007; Krog-Mikkelsen et al., 2011; Raben, 2002; Scazzina et al., 2011). However, in the majority of the studies, where high and low GI carbohydrates have been included in mixed food matrices, no differences have been observed in DIT (Díaz et al., 2005; Kaur et al., 2016; Keogh et al., 2007; Krog-Mikkelsen et al., 2011).

Overweight and obese individuals often quit weight loss diets. One reason for this lack of adherence is increased sensation of hunger (Melby, Paris, Foright, & Peth, 2017; Polidori, Sanghvi, Seeley, & Hall, 2016). Consequently, it is advantageous to investigate if meals with certain combinations of protein source and carbohydrates influence satiety and appetite differently. The objective of the present study was to investigate the acute effects of meals containing cod versus veal in combination with carbohydrates with high or low GI on DIT, appetite, and EI, as well as postprandial ghrelin, glucose, and insulin responses in overweight men and women. Based on available data we hypothesized that cod, in combination with low GI carbohydrates, would increase DIT and satiety compared to cod in combination with high GI carbohydrates and veal in combination with carbohydrates with low and high GI.

2. Methods

2.1. Experimental design

The study was conducted as a randomized, 4-condition, crossover study and was carried out at the Department of Nutrition, Exercise and Sports, University of Copenhagen, from March 2015 to January 2016. On four occasions, minimum one week apart and in the follicular phase of the menstrual cycle for the women, the participants arrived at the laboratory at 7.30 a.m. in a fasting state. Upon arrival, participants were required to attempt to void, and body weight was recorded to the nearest 0.05 kg on an electronic decimal scale (Lindeltronic 8000, Copenhagen, Denmark). Following this, participants laid down, and the non-dominant hand was placed in a heat box. After 15 min of rest in the supine position, blood pressure and temperature were measured and an intravenous catheter was inserted in the back of the heated hand. At 8.15 a.m. a fasting blood sample was drawn, and the first visual analog scales (VAS) were provided to register appetite sensations and gastrointestinal experiences. Thereafter, resting metabolic rate (RMR) was measured using a ventilated hood system (Jaeger Oxycon PRO; Viasys Healthcare GmbH). At 8.55 a.m. the participants were given one of four test meals. The participants were instructed to eat at a constant pace and to distribute the meal over a period of 15 min. After the test meal, VAS on appetite sensations and general palatability of the test meal were completed. During the following 3 h respiratory gas exchanges were measured six times at a 25-min duration with 5-min breaks between each measurement using a ventilated hood system. Blood samples were drawn every 20 min after the test meal, and appetite sensations were assessed by VAS every 30 min until 12.30 p.m. where gastrointestinal experiences also were assessed. After the last blood sample was drawn, the participants were asked to empty their bladder. Thereafter, they were served an *ad libitum* buffet-style lunch. A list of the planned measurements and time points can be found in Supplementary Table 1.

2.2. Subjects

Healthy, overweight men and women were recruited from the Copenhagen area through newspaper ads, the web page www.forsøgsperson.dk, and the website of the Department of Nutrition Exercise and Sports. To be included in the study, participants had to be non-smoking, 18–50 years of age with a body mass index (BMI) of 25.0–30.0 kg/m^2, with fasting blood glucose level below 5.9 mmol/L. Exclusion criteria included: self-reported eating disorders or irregular eating schedule (e.g. skipping breakfast), chronic diseases, use of prescription medication that has the potential to affect body weight or glucose metabolism, psychoactive medication, epileptic medication, or weight loss medications, food allergies, substance abuse, vigorous physical activity of more than 10 h/week, alcohol intake above the recommendations from the Danish Health Authority (7 or 14 units per week for women and men, respectively), daily caffeine intake above 300 mg, night- or shift work, blood donation less than 1 month before study commencement and during study period, or simultaneous participation in other clinical studies. Women who were pregnant, breastfeeding or intended to become pregnant during the study period,

women in the menopausal transition and post-menopausal women, and women with irregular menstrual cycles, were also excluded. The study was conducted in accordance with the Declaration of Helsinki, and all procedures were approved by the Municipal Ethical Committee of the Capital Region, Denmark (journal number H-1-2014-038). The study was registered at clinicaltrials.gov (NCT02770833).

2.3. Standardization

The participants were instructed to eat a standardized 3500 kJ dinner between 7 p.m. and 8 p.m. the night before the test days (a chicken paprika dish served with rice and orange juice: 16.6 E% protein, 50.1 E% carbohydrate and 33.3 E% fat), provided from the department as a frozen product. The participants were required to fast from 8 p.m. They were allowed to drink 500 mL water during fasting, although maximum 250 mL in the morning of the test day. The participants were instructed to abstain from vigorous physical activity, medicine, and alcohol 48 h before the test days. On the test days, participants arrived by transport requiring a minimum of physical activity (by car, bus or train). They were instructed to use the same means of transportation on all four test days.

2.4. Test meals

The test meals consisted of patties of cod served with mashed potatoes (CM), patties of cod served with wholegrain pasta (CP), patties of veal served with mashed potatoes (VM), and patties of veal served with wholegrain pasta (VP). Additionally, all meals were served with tomato sauce and water. The test meals were iso-caloric ~2012 kJ/meal and had an identical macronutrient distribution (approximately 25.5 E% protein, 41 E% carbohydrate and 33.5 E% fat), equivalent energy density and balanced fiber content, see Table 1. The wholegrain pasta and the mashed potatoes had low and high GI of 45 and 87, respectively (Atkinson, Foster-Powell, & Brand-Miller, 2008). Plasma glucose levels in response to the wholegrain pasta and the mashed potatoes were assessed in a pilot study before the main study (Supplementary Fig. 1). The veal originated from conventional Danish calves fed milk, hay, straw, haylage, silage, and concentrate. The calves were slaughtered when a weight of 180–240 kg was reached at a maximum age of 10 months.

Table 1
Recipe and nutritional composition of the test meals.

	CM	CP	VM	VP
Cod filet (g)	115.5	115.5	–	–
Wholegrain pasta (g)	–	45.2	–	45.2
Veal (g)	–	–	100	100
Mashed Potato Powder (g)	48.0	–	48.0	–
Tomato puree concentrate (g)	17.6	17.6	17.6	17.6
Onion (g)	32.0	32.0	32.0	32.0
Table salt (g)	2.4	2.4	2.4	2.4
Whole egg (g)	9.6	9.6	9.6	9.6
Cream 38% (g)	11.2	11.2	11.2	11.2
Breadcrumbs (g)	9.6	9.6	9.6	9.6
Rapeseed oil (g)	6.0	6.0	5.2	5.2
Butter (g)	6.33	6.35	5.5	5.5
Water with meal (g)	225.8	228.8	242.8	246.0
Energy (kJ)	2012	2011	2013	2012
Total weight (g)	484	484	484	484
Density (kJ/g)	4.16	4.15	4.16	4.16
Protein %E	25.1	25.6	25.1	25.6
Fat %E	34.3	33.3	34.3	33.3
Carbohydrate %E	40.6	41.1	40.6	41.1
Fiber (g)	4.8	5.0	4.8	5.0
Glycemic index	87	45	87	45
Glycemic load	40	21	40	21

CM, cod and mashed potatoes; CP, cod and pasta; VM, veal and mashed potatoes; VP, veal and pasta.

2.5. Energy expenditure and substrate oxidation

Energy expenditure (EE) and substrate oxidation were measured by indirect calorimetry with the use of a ventilated hood system (Jaeger Oxycon PRO; Viasys Healthcare GmbH). The precision of the ventilated hood was validated by an alcohol burning test on a weekly basis. Energy expenditure and oxidation of carbohydrate and fat were calculated from the gas exchange using constants of Elia and Livesey (Elia & Livesey, 1992). Protein oxidation was calculated from urinary nitrogen based on analysis of urea (see below). Diet-induced thermogenesis was calculated as area under the EE curve above fasting (RMR) level (kJ/min* min) and was the primary endpoint. Each measurement lasted for 25 min, of which the last 20 min were used for calculation of EE.

2.6. Biochemical analyses

Blood samples were drawn through an indwelling superficial back of the hand catheter, and all blood samples were drawn by the heated-hand box method, a recognized laboratory technical method to obtain 'arterialized'-venous blood (Abumrad, Rabin, Diamond, & Lacy, 1981). The hand was placed in a cavity through which heated air was circulated to warm the hand to 50 °C. Blood for analyses of plasma glucose and lactate was drawn into sodium fluoride-oxalate-prepared tubes. Blood collected for analyses of serum insulin and C-peptide was drawn into serum clot activator tubes. Blood for analyses of plasma ghrelin levels was drawn into EDTA-prepared tubes containing aprotenin. Immediately after collection, blood for analyses of glucose, lactate, and ghrelin was centrifuged (2500 × g for 10 min at 4 °C). Blood samples for insulin and C-peptide analyses were allowed to coagulate for 20 min before centrifugation. All samples were subsequently frozen and stored at −80 °C until they were analyzed. Plasma glucose and lactate were analyzed on an ABX Pentra 400 (plasma glucose: intra CV %: 1.4, inter CV %: 2.5; plasma lactate: intra CV %: 0.2 inter CV %: 0.9). Serum insulin and C-peptide were analyzed on an Immulite 1000 (serum insulin: intra CV %: 4.2 inter CV %: 4.2; serum C-peptide: intra CV %: 2.5 inter CV %: 1.8). Total plasma ghrelin was analyzed with an enzyme-linked immunosorbent assay produced by EDM Millipore, Darmstadt, Germany (intra CV %: 3.8 inter CV %: 8.9).

To assess protein oxidation during the measurements of EE, all urine produced by the participants during the stay at the laboratory on each test day was weighed, and a 10 mL sample was used for analysis of urea (intra CV %: from 0.74 to 1.24 dependent om concentration, inter CV %: from 3.80 to 4.13, dependent on concentration; ABX Pentra 400).

2.7. Visual analog scales

Appetite sensations and evaluations of the test meals and the lunch were assessed by VAS of 100 mm in length. At each end of the line the most negative or positive rating was expressed (Flint, Raben, Blundell, & Astrup, 2000). Participants answered questions regarding hunger, satiety, prospective food consumption (PFC), fullness, and desire to eat something sweet, salty, rich in fat, or meat/fish. The test meals and the buffet-style lunch were rated in regard to palatability, look, odor, off taste and general appearance. In the evaluation of the meals, 0 and 100 corresponded to the most positive and negative ratings, respectively. Additionally, participants answered eight yes/no questions regarding gastrointestinal symptoms (bloating, diarrhea, rumbling, throat burn, flatulence, nausea, acid reflux, and stomach pain). The VAS on gastrointestinal symptoms was filled in before the test meal was ingested, and at 110 and 200 min after the test meal. If the participant had experienced a gastrointestinal symptom, they had to rate the intensity on

a VAS of 100 mm in length.

The VAS questionnaires were provided to the participants using an electronic tablet-based VAS (eVAS). The eVAS system was set up on an HP Slate 2 tablet using Acqui version 1 (xyzt, Copenhagen, Denmark). Prior to this study, we validated the eVAS, and it was found to be comparable to the traditional pen-and-paper method (Supplementary Fig. 2).

2.8. Ad libitum buffet-style lunch

After the last measurement of gas exchange, the participants were presented with an *ad libitum* cold and hot buffet-style lunch. The buffet contained a variety of foods, representing a typical Danish lunch, with a wide selection of food groups. The participants were instructed to eat at a constant pace and to stop eating when they felt pleasantly satiated. All foods were weighed to the nearest gram by an experienced food technician before and at the end of the buffet.

2.9. Blinding and randomization

Eligible participants were randomly assigned to a combination of the four test meals. The study coordinator was responsible for the randomization, which was stratified on men and women, i.e. two separate lists of the 24-meal sequences were generated in an online randomization-program (Research Randomizer, n.d.): one to allocate women and one to allocate men. Blinding of the study was not possible as the appearance and odor of the four test meals could not be concealed.

2.10. Sample size

The sample size calculation was based on a previous study in such a way that 20 subjects would give a statistical power of 90% to detect a difference of 35 kJ in DIT with a within-subject SD of 32 kJ at a 2-sided 5% significance level (Lorenzen, Frederiksen, Hoppe, Hvid, & Astrup, 2012). Moreover, 20 subjects were sufficient to detect a 10 mm difference in satiety, hunger, fullness, and PFC at a statistical power of 90% (Flint et al., 2000).

2.11. Statistical analysis

Baseline data are presented as mean ± SD. Available-case analyses were carried out using data from all subjects until dropout or end of study. Repeated measurements were analyzed using linear mixed models that included a time-meal interaction and were adjusted for sex, age, BMI, visit number, and fasting outcome value on the test day. Overall subject and within-visit subject differences were accommodated using random effects. Serial correlation between repeated measurements on the same subject (within each visit) was modeled assuming a spatial Gaussian correlation structure (exponentially decreasing correlation over time). In case a significant time-meal interaction was found, model-based pairwise comparisons, adjusted for multiple testing, were used to identify time points with differences between meals. Multiplicity adjustment of *P* values was based on the single-step method (Hothorn, Bretz, & Westfall, 2008). Models including summary measures (DIT, iAUC [incremental AUC], iAOC [incremental area over the curve]*), ad libitum* EI, and the evaluation of the test meals included only a main effect of meal (as there were no repeated measurements within visit) and were adjusted for sex, age, BMI, and visit number. Subject was included as random effect. If a significant effect of meal was found, model-based pairwise comparisons, adjusted for multiple testing, were used to identify differences between test meals. DIT, iAUC, and iAOC were calculated by the trapezoidal rule. Models investigating differences in appetite sensations, both as repeated measures, iAOC or iAUC were additionally adjusted for palatability of the test meals. Supplementary Table 2 shows pairwise comparisons for each of the outcomes.

For all models, normality and homogeneity of variance were assessed graphically using residual plots and normal probability plots. Fisher's exact test was used to investigate differences in occurrence of gastrointestinal side effects.

Results are shown as mean ± SE or mean difference between meals ± SE unless otherwise specified. Graphs are based on unweighted average ± SEM. P values < 0.05 were considered significant. All statistical analyses were performed in R version 3.1.2 (R Core Team, 2014).

3. Results

3.1. Subjects

A total of 32 potential participants attended a screening visit at the Department of Nutrition, Exercise and Sports. Three did not meet the inclusion criteria, and five withdrew their informed consent before the first study day. Thus, 24 participants, 16 men, and 8 women were randomized. Twenty-one participants completed all four test days. Two participants dropped out, completing one and two test days, respectively, and one participant never showed up. Baseline characteristics are shown in Table 2.

3.2. Appetite sensations

Postprandial responses in satiety and hunger are shown in Fig. 1. There were no meal-time interactions and no meal effects in any of the appetite parameters (satiety, hunger, fullness, or PFC) (all $P > 0.05$). Furthermore, no differences were found between the meals for appetite parameters summarized as iAUC or iAOC (all $P > 0.05$).

There were neither meal-time interactions nor any meal effects for the desire to eat meat or fish or specific tastes (salty, fatty or sweet), (all $P > 0.05$).

3.3. Ad libitum EI

Ad libitum EI and the macronutrient distribution of ingested food after the four test meals are shown in Table 3. There were no differences between the test meals (all $P > 0.05$).

3.4. Ghrelin

There was a significant time-meal interaction for plasma ghrelin ($P = 0.02$). However, pairwise comparisons adjusted for multiple testing did not reveal any difference between meals at any of the time points (all $P > 0.05$). For ghrelin summarized as iAOC, no differences were found between test meals ($P = 0.46$), see Fig. 2.

3.5. Palatability of the test meals

There were no differences in the evaluation of palatability, look, odor, off taste, or general appearance between the four test meals (all

Table 2
Baseline characteristics of the 24 overweight men and women.[1].

Characteristics	Values
Age, y	30.0 ± 7.6
Weight, kg	87.8 ± 7.5
Height, m	179.5 ± 8.7
BMI, kg/m^2	27.2 ± 1.4
Systolic blood pressure, mmHg	118.1 ± 7.2
Diastolic blood pressure, mmHg	74.6 ± 5.3
Fasting glucose, mmol/L	5.1 ± 0.5

[1] Data are presented as mean ± SD.

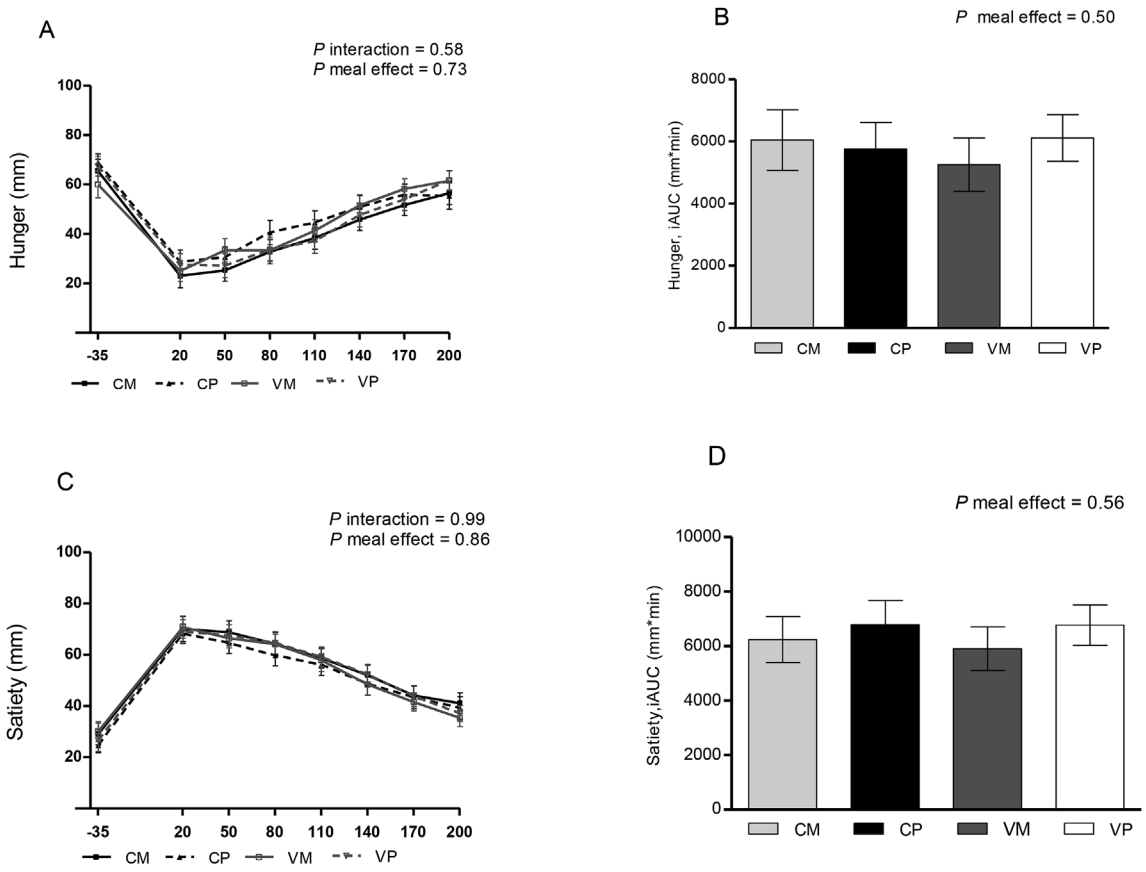

Fig. 1. Mean unadjusted changes in hunger (A) and satiety (C) and corresponding iAOC (B) and iAUC (D) in overweight men and women after intake of the four different test meals: CM, CP, VM, and VP. Data are presented as mean ± SEM, n = 23. Appetite ratings over time were analyzed by repeated measures. iAOC and iAUC were analyzed by mixed linear models. iAUC, incremental area under the curve; iAOC, incremental area over the curve; CM, cod and mashed potatoes; CP, cod and pasta; VM, veal and mashed potatoes; VP, veal and pasta.

Table 3
Ad *libitum* EI after the four different test meals[1,2].

	CM	CP	VM	VP
Total EI, kJ	4884 ± 509	5159 ± 513	5072 ± 510	4943 ± 510
Protein, kJ	798 ± 93	828 ± 95	870 ± 94	857 ± 94
Carbohydrates, kJ	2108 ± 249	2274 ± 250	2167 ± 249	2137 ± 249
Fat, kJ	1975 ± 245	2052 ± 247	2034 ± 244	1946 ± 245

[1] Data are presented as mean ± SE.
[2] No differences between test meals in energy intake or macronutrient distribution. EI, energy intake; CM, cod and mashed potatoes; CP, cod and pasta; VM, veal and mashed potatoes; VP, veal and pasta.

$P > 0.05$), see Table 4.

3.6. DIT and substrate oxidation

There were no differences in DIT between the test meals ($P = 0.12$). Furthermore, no interaction between meal and time or meal effect was found for the postprandial increase in EE over time ($P = 0.44$ and $P = 0.09$, respectively) see Fig. 3. Fat and carbohydrate oxidation were not different between test meals, neither when analyzed as repeated measures ($P = 0.39$ and $P = 0.16$, respectively) nor as iAUC ($P = 0.75$ and $P = 0.50$, respectively). There were, furthermore, no differences in protein oxidation between the test meals ($P = 0.71$).

3.7. Glucose, insulin, C-peptide, and lactate

A meal-time interaction was observed for both plasma glucose ($P = 0.04$) and serum insulin ($P < 0.001$). Pairwise comparisons showed that the meals with mashed potatoes (high GI) resulted in the highest response, see Fig. 4. For glucose summarized as iAUC, the CM meal was found to increase the glucose concentration compared to the CP meal and the VP meal (all $P < 0.05$), see Fig. 3. For insulin summarized as iAUC, the CM and the VM meals were found to induce higher concentrations compared to the CP and the VP meals (all $P < 0.001$), see Fig. 3. A time-meal interaction was observed for lactate ($P = 0.001$) and C-peptide ($P = 0.001$), the meals with mashed potatoes (high GI) resulted in the highest responses (Supplementary Fig. 3).

3.8. Well-being and gastrointestinal side effects

A significant meal effect was found for well-being. Well-being was rated 5.1 ± 1.5 mm lower after the VM meal compared to the VP meal ($P = 0.006$). There were no differences in gastrointestinal feelings (bloatedness, diarrhea, rumbling, throat burn, flatulence, nausea, acid reflux, and stomach pain) at fasting, 110, or 200 min after the test meal (all $P > 0.05$).

4. Discussion

The present study showed no differences in appetite sensations, *ad libitum* EI, and DIT after meals with cod or veal combined with low or high GI carbohydrates. Thus, we were unable to confirm our hypothesis that cod in combination with low GI carbohydrates would increase DIT and satiety compared to cod with high GI carbohydrates and veal with low or high GI carbohydrates.

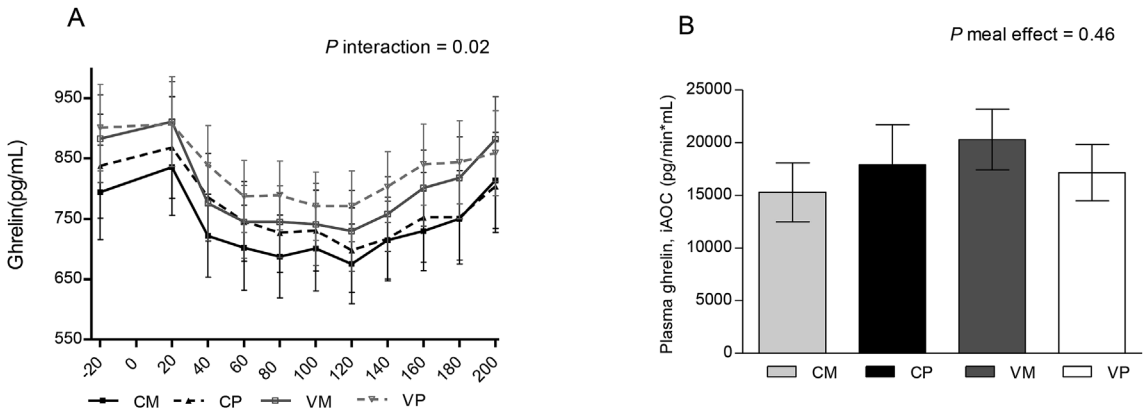

Fig. 2. Mean unadjusted 200-min changes in plasma ghrelin concentrations (A) and corresponding iAUC (B) in overweight men and women after intake of the four different test meals CM, CP, VM, and VP. Data are presented as mean ± SEM, n = 23. Concentrations over time were analyzed by repeated measures. iAOC was analyzed by a mixed linear model. iAOC, incremental area over the curve; CM, cod and mashed potatoes; CP, cod and pasta; VM, veal and mashed potatoes; VP, veal and pasta.

Table 4
Palatability evaluations of the four test meals[1, 2].

	CM	CP	VM	VP
General appearance (mm)	40.5 ± 3.8	44.8 ± 3.9	47.4 ± 3.8	42.2 ± 3.8
Look (mm)	60.4 ± 5.1	58.3 ± 5.1	64.0 ± 510	61.8 ± 5.1
Off taste (mm)	79.0 ± 5.1	76.1 ± 5.1	72.0 ± 5.1	74.0 ± 5.1
Smell (mm)	37.8 ± 4.3	41.0 ± 4.4	36.4 ± 4.3	33.6 ± 4.3
Palatability (mm)	30.8 ± 4.3	34.9 ± 4.3	38.9 ± 4.3	28.3 ± 4.3

[1] Data are presented as mean ± SE.
[2] No differences between test meals in any of the evaluations. mm, millimeters; CM, cod and mashed potatoes; CP, cod and pasta; VM, veal and mashed potatoes; VP, veal and pasta.

We found no differences between the test meals in satiety, fullness, hunger, PFC, or EI at the *ad libitum* buffet-style lunch. Moreover, plasma levels of ghrelin, which is a known orexigenic hormone, did not differ between meals. These results are partly in line with the results from a similar acute meal test study conducted by Borzoei et al. (Borzoei et al., 2006). Here, 23 normal-weight men were served an iso-caloric-macronutrient-balanced beef or cod lunch in a crossover design, and no differences were found in satiety, hunger, or PFC between test meals. However, in contrast to our results, they found a lower EI at the *ad libitum* meal, 4 h after consumption of the cod lunch meal compared to the beef lunch meal. In the present study, the *ad libitum* meal was a buffet-style lunch, whereas a macronutrient-fixed single-course *ad libitum* meal was used in the study by Borzoei et al. The buffet-style *ad libitum* meal has the advantage that it may be more representative of free-living conditions. However, a limitation with the buffet-style *ad libitum* meal is that the wide selection of foods in the buffet is likely to delay satiation, stimulate interest in foods, and increase EI (Blundell et al., 2010). Moreover, the difference in satiating effect of the macronutrients can affect the *ad libitum* EI (Gregersen et al., 2008). In the present study, the participants consumed on average 5537 kJ at the *ad libitum* meal, whereas the mean EI at the *ad libitum* meal in the study by Borzoei et al. was 2805 kJ after the cod lunch meal and 3133 kJ after the beef lunch meal. Thus, difference in the design of the *ad libitum* meal may be responsible for the discrepancies between the results on *ad libitum* EI in the study by Borzoei et al. and the present study with a buffet-style lunch likely to favor an increased EI. The energy percentage from protein in the test lunch meals was, furthermore, high in the study by Borzoei et al. (47 E% protein), and the authors suggest that the observed effect might not have been seen if lower protein content had been applied. As the protein content in our study was 26 E% compared to the 47 E% in the study by Borzoei et al., this might also explain discrepancies between the two studies.

We found no differences in appetite sensations or *ad libitum* EI between the meals with high GI and low GI carbohydrates. This is in agreement with the majority of studies that have investigated the effect of high and low GI carbohydrates in mixed meals (Díaz et al., 2005; Kaur et al., 2016; Keogh et al., 2007; Krog-Mikkelsen et al., 2011).

The greater thermic effect of protein compared with carbohydrate and fat has been proposed as a mechanism for protein-induced satiety (Westerterp-Plantenga et al., 1999). Research on the effect of different protein sources on DIT is limited, and the results are inconclusive. Furthermore, most studies are limited to examining soy and dairy proteins (Acheson et al., 2011; De Cássia, Alfenas, Bressan, & Cardoso DePaiva, 2010; Lorenzen et al., 2012; Tan, Batterham, & Tapsell, 2010). To the best of our knowledge, only one study, conducted by Soucy and LeBlanc in 1998, has investigated the effect of fish (Soucy julie, 1998). They investigated VO_2 in response to 195 g beefsteak and 250 g cod fillet (both containing 43 g protein). Data were analyzed in two phases, and the authors found a greater mean AUC of VO_2 in the phase from 10 to 40 min after intake of the beef steak compared to the cod fillet, whereas no difference was observed in the phase from 90 to 180 min. However, the beef and cod servings had different weights, and the authors did not provide information on the energy content in the servings. This, together with methodical differences, makes comparisons to the present study difficult. Mikkelsen et al. found a 2% increase in 24-h EE after 4 days on an intervention diet with pork-meat-protein compared to a soy-protein-diet (Mikkelsen, Toubro, & Astrup, 2000). They suggested that the higher content of histidine (27%), methionine (14%), and cysteine (14%) in the pork compared to the soy diet may have resulted in increased protein synthesis and protein turnover rate after intake of the pork diet and thereby a higher EE. Veal and cod differ in the content of these amino acids. Based on data from the Danish food composition database, the 100 g veal in our present study contained a 38% higher amount of histidine, and an 11% higher amount of cysteine compared to the 115.5 g cod, whereas the 115.5 g cod contained a 30% higher amount of methionine compared to the 100 g veal ("Frida Food Data, Release 3.," 2017). However, differences in amino acid composition between the two protein sources did not translate into a difference in DIT in our study.

We found no differences in DIT between the meals with high GI and low GI carbohydrates. This is consistent with the majority of the studies which have investigated the acute effect of mixed meals with low and high GI carbohydrates on DIT (Díaz et al., 2005; Kaur et al., 2016; Keogh et al., 2007). Diaz et al. investigated the effect of macronutrient-balanced breakfast and lunch meals with either high or low GI carbohydrates and found no difference between the meals in either 5-h DIT after the breakfast meals or in 4.5-h DIT after the lunch meals (Díaz

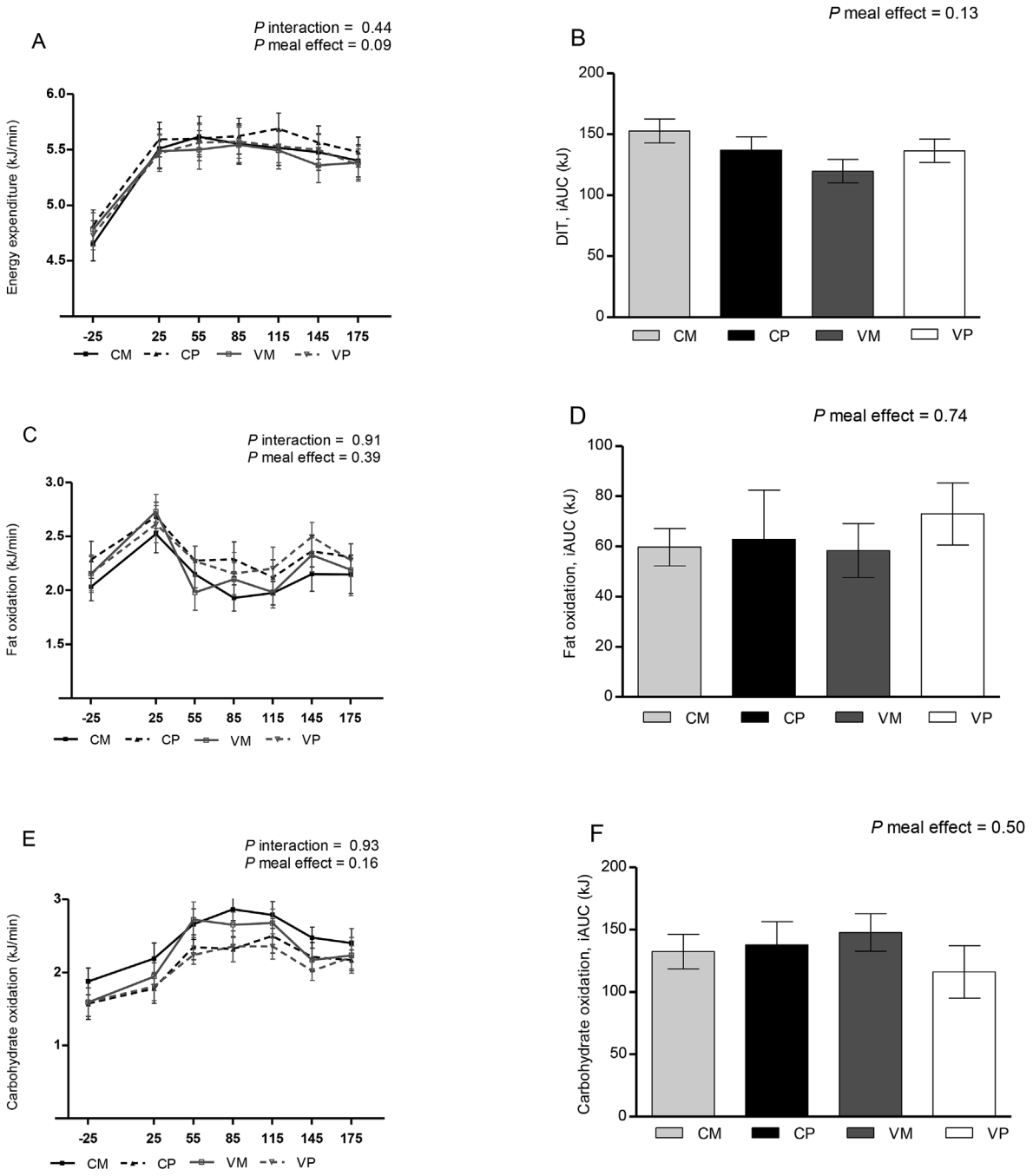

Fig. 3. Mean unadjusted changes in EE (A) and substrate oxidation (C, E) and corresponding iAUCs (B, D, F) in overweight men and women after intake of the four different test meals: CM, CP, VM, and VP. Data are presented as mean ± SEM, n = 23, as both repeated measures and iAUC analyzed using mixed linear models. EE: energy expenditure; DIT, diet-induced thermogenesis; iAUC, incremental area under the curve; CM, cod and mashed potatoes; CP, cod and pasta; VM, veal and mashed potatoes; VP, veal and pasta.

et al., 2005). On the contrary, Scazzina et al. found an increased 8-h DIT after a meal with low GI (biscuit bar with jam) compared to a meal with high GI (bread and jam) (Scazzina et al., 2011). However, the authors did not provide information regarding fiber content of the meals. Thus, it is uncertain if the difference can be ascribed to GI *per se*. Furthermore, the meals had low energy percentages from protein (8.6 E% in the high GI and 6.5 E% in the low GI meal), whereas the test meals in the present study and the studies that did not show any difference in DIT between low and high GI meals had protein contents varying from 12 E% to 25.5 E% (Díaz et al., 2005; Kaur et al., 2016; Keogh et al., 2007; Krog-Mikkelsen et al., 2011). Thus, it could be speculated whether the higher protein content could have masked a potential difference in DIT between low and high GI carbohydrates.

In the present study, plasma glucose, lactate, serum insulin, and C-peptide varied in response to the GI of the carbohydrates, but not the protein source. However, the acute effect of lean fish on glucose metabolism may not be reflecting the effect of a longer term habitual intake, as the daily inclusion of lean seafood compared to lean meat has been shown to improve glucose and lipid metabolism after 4 weeks in healthy men and women (Aadland et al., 2016).

The results from the present study should be interpreted taking the following strengths and limitations into account. Strengths include the randomized crossover study design and our comprehensive standardization procedures. The test meals were iso-caloric and macronutrient-

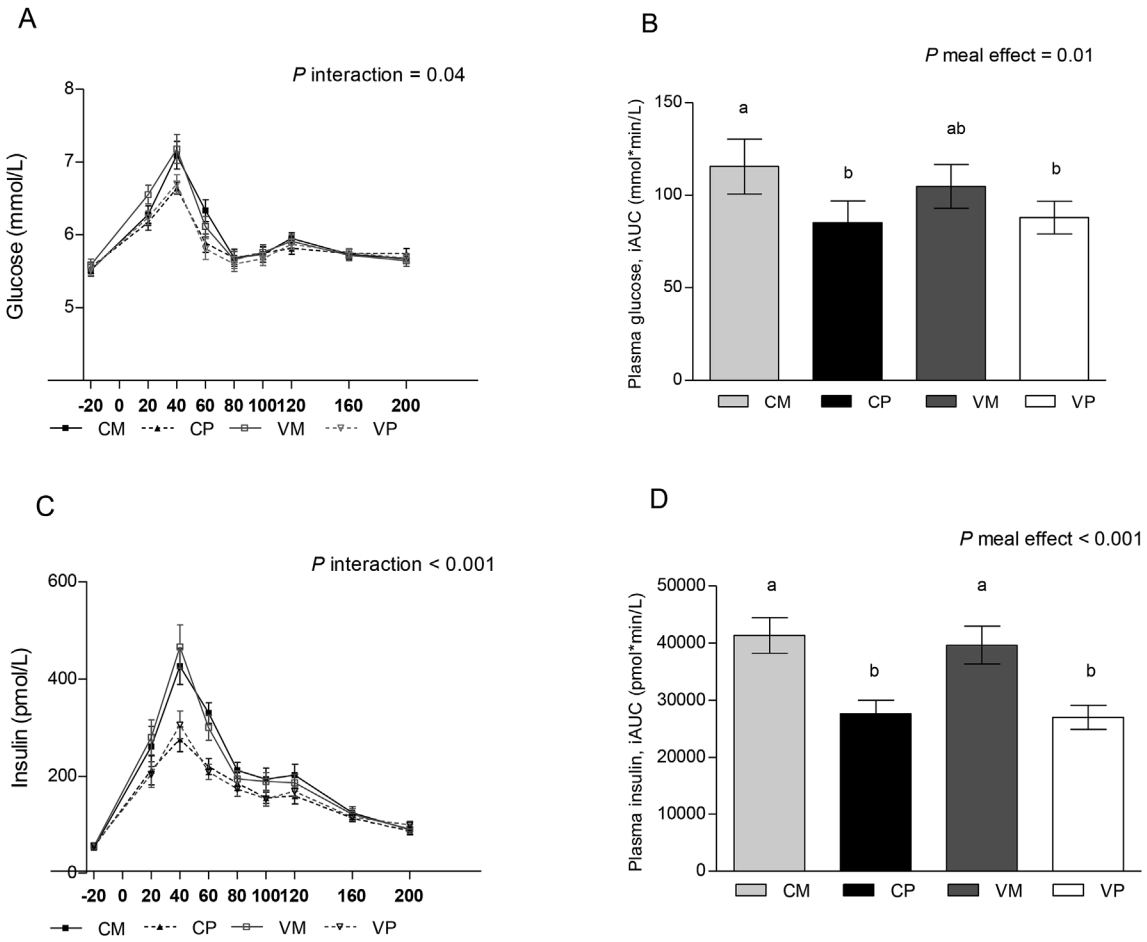

Fig. 4. Mean unadjusted 200-min changes in plasma glucose (A) and serum insulin (C) concentrations and corresponding iAUCs (B, D) in overweight men and women after intake of the four different test meals: CM, CP, VM, and VP. Data are presented as mean ± SEM, n = 23. Concentrations over time were analyzed by repeated measures. Post-hoc model-based pairwise comparisons were adjusted for multiple testing; Glucose: 20 min VM > VP, 40 min VM > VP, CP; CM > CP, VP; 60 min CM > VP, CP (all $P < 0.05$). Insulin: 20 min VM > VP, CP; 40 min VM > VP, CP; CM > CP; VP; 60 min; VM > VP, CP; CM > CP, VP (all $P < 0.05$). iAUC was analyzed by mixed linear models, means not sharing a common letter differ. iAUC, incremental area under the curve; CM, cod and mashed potatoes; CP, cod and pasta; VM, veal and mashed potatoes; VP, veal and pasta.

balanced, and had the same weight and fiber content. Furthermore, the inclusion of the protein sources in whole meals with low and high GI carbohydrates, which allowed the detection of the possible influence of the accompanying type of carbohydrate, is unique, and has, to the best of our knowledge, not been previously conducted. In meal test studies, differences in rating of palatability between test meals are often a problem (Kehlet et al., 2017; Kristensen, Bendsen, Christensen, Astrup, & Raben, 2016; Nielsen et al., 2018). A strength of the current study is, therefore, that there were no differences in the subjective evaluation of the test meals. However, a weakness was demonstrated as well-being was rated lower after the VM meal compared to the VP meal. Preferably, well-being should be rated similarly bewteen all test meals. Women were tested in the follicular phase of their menstrual cycle. However, EI is similarly high during the early follicular phase and the luteal phase and decreases during the follicular phase to a minimum in the periovulatory phase (Asarian & Geary, 2013). This could, therefore, have affected our results on appetite sensations and EI. A further weakness of the present study is that total ghrelin, which represents both acylated and deacylated forms, was measured and not acylated ghrelin, as only acylated ghrelin stimulates appetite (Singhal, Misra, & Klibanski, n.d.).

Diet-induced thermogenesis was, in the present study, measured for 3 h and 20 min. According to a study by Reed and Hill, the optimal period for measuring DIT is 6 h even for meals with an energy content below 3400 kJ (Reed & Hill, 1996). Thus, a 3-h measurement of DIT can, according to Reed and Hill, miss more than 40% of total DIT, and a 4-h measurement of DIT can miss 22.5% of total DIT. Energy expenditure, in the present study, did not return to baseline. Thus, a longer duration of the measurement could potentially have influenced our results. However, a vast part of DIT takes place the first hours following ingestion (Reed & Hill, 1996; Weststrate, 1993). Consequently, we assume that the measuring period used in the present study constitutes a valid measure of DIT. Furthermore, the measuring period of 3 h and 20 min was chosen to obtain an optimal timing of the *ad libitum* meal. If the *ad libitum* meal had been served 6 h after the test meals, it is possible that potential differences between the effects of test meals on EI might have diminished.

Although acute trials are of high value, it is not possible to assess whether the lack of differences between the test meals in the present study will persist over a longer period. In the DIOGENES trial an *ad libitum* diet with a combination of modestly higher protein and lower glycemic index (GI) was shown to result in more effective weight maintenance after weight loss when compared with the official dietary guidelines (Larsen et al., 2010). A subsequent analysis of the source of the dietary proteins revealed no differences in weight maintenance with a higher intake of plant protein and a proportional decrease in animal protein. However, a higher plant protein from cereal products at the cost of non-cereal protein was associated with a larger increase in body weight (van Baak et al., 2017). Neacsu et al. investigated the effect of a vegetarian- or a meat-based high-protein weight loss diet and found no

difference in weight loss or appetite sensations between the diets. However, the participants did only consume the diets for 2 weeks and it is, consequently, not possible to assess whether the impact on weight loss and appetite sensations will remain the same over a longer period (Neacsu, Fyfe, Horgan, & Johnstone, 2014). Krog-Mikkelsen et al. investigated the effect of two *ad libitum* high carbohydrate diets with either low GI carbohydrates or high GI carbohydrates and found that fullness was higher in the low GI group compared with the high GI group after 10 weeks on the *ad libitum* diets (Krog-Mikkelsen et al., 2011). However, there were no differences in hunger, satiety, PFC, or DIT. Aston et al. found, furthermore, no difference in appetite or EI in obese women after 12 weeks on high GI and low GI diets (Aston, Stokes, & Jebb, 2008). The effect of a longer-term diet with high and low GI carbohydrates in combination with protein from different sources has, to the best of our knowledge, not been investigated.

In conclusion, no differences were found in appetite sensations, *ad libitum* EI, or DIT between macronutrient-balanced meals with cod or veal in combination with carbohydrates with low or high GI. However, the meals with high GI carbohydrates induced higher glucose and insulin responses compared to the meals with low GI independently of protein source.

Conflicts of interest

LVN, SN, LK, NJ, JR, CR, BL, KK, LM and AR, declare no conflicts of interest.

Sources of financial support

This work was supported by the Norwegian Seafood Research Fund [FINS 900842].

Acknowledgements

The authors are grateful for the valuable assistance from Søren Andresen, Eivind Bjørås, John Gargul Lind, Charlotte Kostecki, and Karina Graff Rossen at the Department of Nutrition, Exercise and Sports, University of Copenhagen. The authors' responsibilities were as follows: SN, AR, and LK designed the study. LVN, JR, and NJ conducted the study. LVN and CR analyzed data, and LVN wrote the manuscript and has primary responsibility for the final content. LM, BL, KK, CR, and AR supplied valuable knowledge throughout the study and co-edited the manuscript. All authors participated in the discussion of the results, reviewed the manuscript and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.appet.2018.08.006.

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