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Lean Seafood Intake Reduces Postprandial C-peptide and Lactate Concentrations in Healthy Adults in a Randomized Controlled Trial with a Crossover Design[1–3]

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Abstract

Background: Recently we showed that lean seafood consumption reduced circulating triacylglycerol (TG) and VLDL concentrations and prevented an elevated total-to-HDL-cholesterol ratio relative to intake of a nonseafood diet.

Objective: We aimed to elucidate whether diet-induced altered carbohydrate metabolism could be a contributing factor to the previously observed different lipoprotein patterns.

Methods: This was a secondary outcome and explorative randomized controlled trial with a crossover design in 20 healthy adults (7 men and 13 women) that were 50.6 ± 3.4 (mean ± SEM) y old, weighed 75.7 ± 2.5 kg, and had a body mass index (BMI, in kg/m^2) of 25.6 ± 0.7. After a 3-wk run-in period and separated by a 5-wk wash-out period, the participants consumed 2 balanced diets [in percentage of energy (energy%); 29% fat, 52% carbohydrates, 19% protein] for 4 wk. The diets varied in the main protein sources; 60 energy% of total protein was from either lean seafood or nonseafood sources. On the first and last day of each diet period, fasting and postprandial blood samples were collected before and after consumption of test meals (in energy%; 28% fat, 52% carbohydrates, 20% protein) with cod or lean beef.

Results: The diets did not alter serum insulin and glucose concentrations. However, relative to the nonseafood diet period, the lean seafood diet period reduced postprandial C-peptide (P = 0.04) and lactate (P = 0.012) concentrations and fasting and postprandial TG/HDL-cholesterol ratios (P = 0.002). Hence, different postprandial lactate levels occurred at equal glucose concentrations.

Conclusions: Even though the diets did not alter serum insulin and glucose concentrations, intake of the lean seafood compared with the nonseafood diet reduced postprandial concentrations of C-peptide and lactate and the TG/HDL-cholesterol ratio in healthy adults in a manner that may affect the long-term development of insulin resistance, type 2 diabetes, and cardiovascular disease. This trial was registered at www.clinicaltrials.gov as NCT01708681. *J Nutr* 2016;146:1027–34.

Keywords: lean seafood, type 2 diabetes, glucose metabolism, postprandial, dietary protein sources, lactate, insulin sensitivity

Introduction

The pandemic of type 2 diabetes (T2D) is an increasing public health burden worldwide (1, 2), and identifying strategies to prevent diabetes is thus warranted. Lifestyle interventions may prevent or postpone the development of T2D (3–6). For instance, adherence to certain dietary patterns, such as the Mediterranean diet, is associated with both a lower risk of T2D development and improved T2D management (7). The Mediterranean diet encourages ≥3 servings of fish (100–150 g) or shellfish (200 g) each week (8), but studies aiming to investigate the association between seafood intake and propensity to develop T2D are inconclusive (9, 10). On the other hand, observational studies indicate that increased consumption of red meat is associated with elevated risk of developing T2D (11).

Data from both animal feeding studies and human intervention studies suggest that lean seafood intake may prevent

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perturbations in glucose homeostasis and insulin resistance and thereby T2D development. In rats fed high-fat diets, a lean fish protein hydrolysate, compared with casein, reduced plasma glucose and insulin concentrations and increased the hepatic glycogen concentration (12). Also, rats fed high-sucrose diets with cod had reduced fasting and postprandial glucose and insulin responses and increased peripheral insulin sensitivity compared with rats fed casein (13). Furthermore, intake of cod protein, relative to soy protein or casein, prevented development of skeletal muscle insulin resistance (14) by normalizing downstream insulin receptor signaling in skeletal muscle of obese rats (15).

In overweight adults, 8 wk of daily cod protein supplement, compared with placebo, reduced fasting glucose, 2-h postprandial glucose, and glucose AUC (16). A previous study showed that 4 wk of cod consumption improved insulin sensitivity in subjects that were insulin resistant at start of the study (17). These observations are further strengthened by randomized controlled trials, demonstrating that inclusion of lean fish, as opposed to other animal proteins, increased sex-hormone binding globulin (18) and HDL_2 concentrations (18, 19), thereby indicating improved insulin sensitivity (20).

A recent prospective population-based cohort study of Norwegian women concluded that a daily intake of 75–100 g of lean fish reduced the risk of developing T2D by ~30% (21). Furthermore, a test meal with cod protein, compared with milk protein, reduced the postprandial insulin levels and lowered the insulin-to-C-peptide and the insulin-to-glucose ratios in healthy women (22). Hence, randomized controlled trials investigating the potential protective effect of lean seafood consumption on T2D development in healthy subjects are warranted.

In a previous paper, we showed that 4 wk of lean seafood intake, compared with nonseafood intake, prevented elevations in postprandial TG, VLDL particle numbers, and total-to-HDL-cholesterol ratio in healthy adults (23). Because there is a strong association between glucose metabolism and dyslipidemia (24), we aimed to study postprandial glucose metabolism. The present study is a combined secondary outcome (postprandial glucose) and explorative (measures related to glucose metabolism and insulin sensitivity) study. We hypothesized that an altered postprandial glucose metabolism contributed as an underlying factor to the observed differences in lipoprotein pattern that we reported previously (23). Changes in glucose metabolism may be associated with reduced insulin sensitivity, and an early marker of reduced insulin sensitivity is elevated pancreatic insulin secretion (25). Because insulin and C-peptide are secreted from the pancreas at a ratio of 1:1 and because the half-life of circulating C-peptide is 2–5 times longer than the half-life of insulin (26–28), we included measurement of both insulin and C-peptide in the present study. Moreover, because an elevated ratio of circulating TG/HDL cholesterol is an acceptable surrogate measure for insulin resistance in white subjects (29–31), calculation of this ratio was included in the study.

Methods

The present data were obtained from a study described in detail previously (23). Participants were recruited during October and November 2012. Diet period 1 was in January–February 2013, and diet period 2 was in April–May 2013. Lunches and dinners in the 2 intervention periods were prepared and served at the Bergen University College. The test-meal servings and blood samplings were done at the Center for Clinical Studies in Bergen, Norway. The study was performed in accordance with the ethical standards of the regional committee on human experimentation. The Regional Committee for Medical and Health Research Ethics of Western Norway (Reference 2012/1084) approved the protocol, informed consent and advertisements. All subjects signed a written informed consent. The study was registered at www.clinicaltrials.gov (NCT01708681).

Subjects. The details of the inclusion/exclusion criteria, as well as the baseline characteristics of the participants, have been described in detail elsewhere (23). This study was conducted in healthy subjects, and before the study the participants were invited to a prestudy visit. The purposes of this prestudy visit were (*1*) to obtain written informed consent from the participants, (*2*) to get information about their habitual food intake through a food-frequency questionnaire in order to make experimental diets at energy levels covering the energy demand of the study population, and (*3*) to ensure that the subjects included in the study were healthy. Hence, at the prestudy visit the physician registered anthropometric measures, blood pressure, heart rate, and conducted a brief examination of the heart and stomach. Fasting blood samples were taken to determine blood hematology and blood glycated hemoglobin (Hb1Ac) and serum concentrations of lipids, glucose, insulin, thyroid-stimulating hormone, and markers of kidney and liver functions. A flow chart for the recruitment, randomization, and data sampling from the participants is shown in **Supplemental Figure 1**. The study population was 50.6 ± 3.4 y old, weighed 75.7 ± 2.5 kg, and had a BMI (in kg/m^2) of 25.6 ± 0.7 (means ± SEMs).

Experimental design and diets. A detailed description of the experimental design, randomization of the study participants, and the experimental diets has been published previously (23). The study was a crossover design with 2 experimental periods of 4 wk separated by a 5-wk washout period (**Supplemental Figure 2**). Before each dietary period, the participants underwent a 3-wk run-in period. During the run-in periods, the participants were given written information to maintain their habitual lifestyle and to follow a diet in accordance with the Norwegian dietary recommendations (32), with additional specifications to include a maximum of one fatty fish (salmon, trout, mackerel, or herring) meal/wk. During the last week of the run-in periods and throughout the experimental periods, the subjects were instructed to avoid alcohol, chocolate or candy, industrial baked cakes or cookies, fast food, probiotics, fish, and fish oil supplements. No monitoring of the diet was implemented during the run-in periods.

Two 7-d experimental diets, one lean seafood and one nonseafood, were composed for the 10,900 kJ/d energy level. Based on the composition of the 10,900 kJ/d energy level, 6 different energy levels (7500–13,500 kJ/d) were established for each experimental diet by reducing or increasing the amount of all ingredients used in that particular diet. The experimental diets were balanced, with the exception of cholesterol levels (23). At the 10,900 kJ/d energy level, the mean cholesterol content was 288 ± 19 mg/d for the lean seafood diet and 394 ± 119 mg/d for the nonseafood diet. The different cholesterol levels were due to 1 omelet lunch in the nonseafood diet, and without this meal, the mean cholesterol value for the other 13 meals in the nonseafood diet was 273 ± 20 mg/d. For the 10,900 kJ/d energy level, the mean fiber content in the experimental diets was 35 g of insoluble fiber (AOAC) and 8 g of soluble fiber (AOAC). The higher endogenous content of EPA and DHA in the lean seafood diet was balanced for by adding cod liver oil to the sauce of each dinner meal in the nonseafood diet before serving (23). Both diets had a concentration of 0.82 g EPA + DHA/d for the 10,900 kJ/d energy level. The daily intake of EPA + DHA during the diet periods was relatively high. However, the age-adjusted mean intake of total fish for women living in Western Norway has been estimated to be 63 g/d, of which 42 g is lean fish and 16 g is fatty fish (33). By using average concentrations of 2.5 mg EPA + DHA/g in lean fish and 25 mg EPA + DHA/g in fatty fish, the mean intake of EPA + DHA from fish was estimated to be ~0.5 g/d for these women.

At the start of dietary period 1, the participants received experimental diets at the energy levels closest to their habitual intake levels (23). During the experimental periods, the participants consumed 7-d rotating experimental diets that contained, in percentage of energy (energy%), 29 energy% fat, 52 energy% carbohydrates, and 19 energy% protein. In the experimental diets, 11.4 energy% of the dietary proteins (60% of total proteins) was from either lean seafood (cod, pollock, saithe, and

scallop) or from nonseafood (breast filets without skin from chicken and turkey, lean beef, lean pork, egg, and low-fat dairy products) sources (23). For the 10,900 kJ/d energy level, the participants consumed 2235 g lean seafood/wk and 1635 g meat/wk, of which 415 g was lean beef and 250 g was lean pork. During the weekdays, the participants completely consumed their dinner meals under supervision. For the rest of the dietary-period meals, subject compliance was assessed daily by an oral questionnaire, and deviations from the diets were noted; however, only minor deviations occurred. A more detailed description of the meal servings in the dietary periods has been published previously (23).

On the first (day 1) and last (day 28) days of the dietary periods, balanced test meals that only differed in the source of experimental proteins, cod or lean beef (23), were served to the participants. The energy distribution of the test meals were 28 energy% fat, 52 energy% carbohydrates, and 20 energy% protein. The test meal size was 2250 kJ for women and 3000 kJ for men. The nonfiber carbohydrate content of the test meals was 64 g for women and 85 g for men. To minimize the contribution of physical activity on our postprandial outcome measures, the subjects were resting, mainly sitting in chairs, on the days of test-meal ingestions and blood samplings. They were instructed to walk calmly to neighboring rooms for test-meal serving, body composition measurement, blood samplings, and toilet visits. The subjects were allowed to drink water during the 6 h after ingestion of the test meal.

Body composition measurement, blood sampling, and analyses. At days 1 and 28 of each diet period, the body composition (lean mass, fat mass, and fat %) of the subjects were determined by DXA in a Hologic Discovery A (S/N 70602) DXA model at the Center for Clinical Studies in Bergen, Norway. At days 1 and 28 of each intervention period, blood samples were drawn after an overnight fast. After a fasting blood sample (−15 min), the subjects ingested the test meals within 15 min, and postprandial blood samples were taken immediately after (0 min) and at 30, 60, 120, 240, and 360 min after completion of the test meal.

Conventional reagents were used to analyze fasting and postprandial concentrations of serum glucose (Roche/Modular P Analyzer), serum insulin, and C-peptide (Siemens/Immulite 2000 Analyzer). Concentration of glucagon in fasting and postprandial EDTA plasma samples (to which Trasylol/Aprotinin was added) was analyzed by a radioimmunoassay (Millipore). Fasting concentrations of serum transferrin receptors (Cobas 8000-e702) and ferritin (Cobas 8000-e602) were analyzed with conventional reagents. The above analyses were conducted at the Laboratory of Medicine and Pathology of Haukeland University Hospital. Fasting and postprandial concentrations of serum nonesterified fatty acids (NEFAs; DIALAB), glycerol, 3-hydroxy-butyrate, OH-butyrate (both Randox), lactate (Spinreact), and urea (MaxMat) were analyzed by conventional enzymatic kits by using a MaxMat PL II chemistry analyzer at the National Institute of Nutrition and Seafood Research. Serum total adiponectin concentration was determined by a radioimmunoassay (Millipore) at the National Institute of Nutrition and Seafood Research. The technicians performing the analyses were blinded by giving the samples unique number codes.

Calculations. From the data in our previous paper (23), we calculated the TG/HDL-cholesterol ratio, as a proxy for insulin sensitivity (29–31). We calculated the ratio of transferrin receptors to ferritin, because an increase in this ratio is associated with increased risk of T2D in healthy women (34).

Fasting surrogate measures of insulin sensitivity was calculated by using the revised quantitative insulin sensitivity check index (35) formulation, 1/{log [glucose (mg/dL)] + log [insulin (μU/mL)] + log [NEFAs (mmol/L)]}. Also, the Disse index (36) was calculated by using the following formula: $12 \times [2.5 \cdot (\text{HDL cholesterol/total cholesterol}) - \text{NEFAs}] - \text{fasting insulin}$. In addition, we assessed insulin sensitivity by the HOMA1-IR (homeostatic model assessment) equation by using the formula HOMA-IR = [(fasting serum glucose, mmol/L · fasting serum insulin, mU/L)/22.5] (37).

Statistical analyses. Sample size was calculated based on the efficacy of fish protein to reduce plasma VLDL-TG as previously described in detail (23). Statistical analyses were performed by using the Statistical Analysis System (version 9.3; SAS Institute). The PROC MIXED procedure for an ANOVA for crossover design with 2 periods as described by Hills and Armitage (38) was used to compare the effects of the 2 dietary treatments on anthropometric measurements and glucose parameters. Because no effect of experimental period or diet sequence and no residual effect of the first experimental period over the second period were observed for any of the measured variables, the data for experimental period, diet sequence, and dietary treatment were pooled. Standard Bonferroni correction has been performed to reduce the chances of obtaining false-positive results. The Bonferroni correction was done by multiplying the original P values by 2 for the fasting and postprandial data, by 3 for the body composition values, and by 3 for the insulin sensitivity indexes because the variables were correlated with each other within these 3 subgroups. Furthermore, repeated-measures analysis of variance was applied for variables measured periodically during the test meal. The least squares means test was performed to compare the changes during the 4-wk diet periods (after − before measures) for both of the diet periods. For all measures, $P < 0.05$ was considered as statistically significant. Data are expressed as means ± SEMs.

Results

No diet effect on body composition. The changes in lean and fat mass were similar during the 2 diet periods (**Table 1**).

Lean-seafood intake reduced fasting TG/HDL-cholesterol ratio. Based on the changes (after − before the 4-wk diet period measures) during both periods, we calculated measures previously associated with insulin sensitivity. The TG/HDL-cholesterol ratio decreased by 13% during the lean-seafood diet period but increased by 30% during the nonseafood diet period, resulting in a significant relative difference between the 2 diets ($P = 0.002$) (Table 1). No change during the diet period was observed for the revised quantitative insulin sensitivity check index, the Disse index, or the HOMA1-IR (Table 1). There was a relative decrease during the diet periods in fasting serum transferrin receptors concentration after the lean-seafood diet compared with after the nonseafood diet, but no diet effect on changes in fasting serum ferritin or ratio of transferrin receptors (TFR) to ferritin was observed (Table 1).

Lean-seafood intake reduced postprandial C-peptide and lactate concentrations and the TG/HDL-cholesterol ratio. We observed no effect on changes during the diet periods for fasting and postprandial serum glucose or insulin concentrations (Table 1, **Figure 1**A, B). The fasting C-peptide concentration was not changed during the diet periods; however, the lean seafood diet period reduced the postprandial serum C-peptide concentrations ($P = 0.04$), relative to the nonseafood diet period (Table 1, Figure 1C). We observed no changes during the diet periods on fasting or postprandial glucagon concentrations (Table 1, **Supplemental Figure 3**A) or on fasting or postprandial adiponectin concentrations (Table 1, Supplemental Figure 3B).

Even though no change during the diet periods was observed for fasting lactate concentration, we observed a change during the diet periods on the postprandial lactate concentration ($P = 0.012$), which was lower after the lean seafood than after the nonseafood diet period (Table 1, Figure 1D). We observed no change during the diet periods on fasting and postprandial NEFAs, glycerol, OH-butyrate, or urea concentrations, but the change during the diet period in postprandial urea concentration tended to be higher after the nonseafood diet period than after the

TABLE 1 Body composition and fasting serum concentrations of metabolites, hormones, and surrogate measures of insulin sensitivity in healthy adults before and after 4-wk periods of consuming lean seafood and nonseafood diets in a crossover design[1]

	Lean seafood diet			Nonseafood diet			
	Pre	Post	Change	Pre	Post	Change	*P* (change)[2]
Body composition							
Lean mass, kg	52.4 ± 2.2	52.1 ± 2.3	−0.3 ± 0.2	53.1 ± 2.3	52.7 ± 2.3	−0.4 ± 0.1	1.00
Fat mass, kg	21.0 ± 1.3	19.9 ± 1.3	−1.1 ± 0.1	21.4 ± 1.2	20.4 ± 1.2	−1.0 ± 0.2	1.00
Fat, %	28.8 ± 1.7	27.8 ± 1.7	−1.0 ± 0.2	28.2 ± 1.7	27.3 ± 1.7	−0.9 ± 0.3	0.81
Metabolites, mmol/L							
Glucose	5.11 ± 0.11	5.01 ± 0.09	−0.10 ± 0.08	5.03 ± 0.13	5.00 ± 0.13	−0.03 ± 0.09	1.00
Lactate	1.50 ± 0.08	1.35 ± 0.07	−0.15 ± 0.08	1.38 ± 0.08	1.41 ± 0.08	0.03 ± 0.07	0.20
Glycerol	0.08 ± 0.01	0.06 ± 0.01	−0.02 ± 0.02	0.06 ± 0.01	0.05 ± 0.00	−0.01 ± 0.01	1.00
NEFAs	0.59 ± 0.06	0.51 ± 0.04	−0.08 ± 0.06	0.54 ± 0.04	0.52 ± 0.04	−0.02 ± 0.06	1.00
OH-butyrate	0.11 ± 0.04	0.11 ± 0.03	−0.05 ± 0.05	0.11 ± 0.03	0.11 ± 0.02	−0.05 ± 0.03	0.84
Urea	6.41 ± 0.44	6.29 ± 0.26	−0.12 ± 0.26	6.52 ± 0.38	7.01 ± 0.23	0.49 ± 0.23	0.16
Hormones and C-peptide							
Insulin, pmol/L	41.5 ± 5.00	33.4 ± 4.66	−8.13 ± 4.94	46.2 ± 5.74	42.8 ± 4.89	−3.38 ± 5.87	1.00
C-peptide, nmol/L	0.61 ± 0.06	0.57 ± 0.05	−0.04 ± 0.03	0.61 ± 0.05	0.64 ± 0.05	0.03 ± 0.03	0.42
Glucagon, pmol/L	28.0 ± 1.58	27.5 ± 0.95	−0.48 ± 1.05	28.3 ± 1.15	29.0 ± 1.44	0.70 ± 0.90	0.86
Adiponectin, ng/mL	22.9 ± 2.55	18.0 ± 1.91	−4.89 ± 1.19	21.4 ± 2.44	16.8 ± 1.90	−4.59 ± 0.85	1.00
Iron metabolism, mg/L							
TFR	3.02 ± 0.19	2.92 ± 0.16	−0.10 ± 0.09	2.94 ± 0.18	3.02 ± 0.15	0.08 ± 0.10	0.04
Ferritin	0.12 ± 0.02	0.11 ± 0.02	−0.01 ± 0.01	0.12 ± 0.02	0.11 ± 0.02	−0.01 ± 0.01	1.00
Surrogate measures of insulin sensitivity							
TG/HDL-C ratio	0.72 ± 0.09	0.62 ± 0.06	−0.10 ± 0.05	0.59 ± 0.07	0.77 ± 0.08	0.18 ± 0.05	0.002
Revised QUICKI	0.41 ± 0.01	0.44 ± 0.02	0.03 ± 0.02	0.42 ± 0.02	0.42 ± 0.01	0.00 ± 0.01	0.18
Disse index	−3.94 ± 1.28	−1.51 ± 1.18	2.43 ± 1.34	−3.64 ± 1.64	−3.35 ± 1.16	0.29 ± 1.36	0.99
HOMA-IR	1.61 ± 0.20	1.25 ± 0.18	−0.36 ± 0.25	1.79 ± 0.25	1.64 ± 0.22	−0.14 ± 0.25	1.00
TFR/ferritin ratio	51 ± 12	64 ± 21	12 ± 13	52 ± 15	65 ± 21	12 ± 8	1.00

[1] Values are means ± SEMs; $n = 20$ (lean seafood) and $n = 19$ (nonseafood). Glucagon was measured in plasma. HDL-C, HDL cholesterol; NEFA, nonesterified fatty acid; Post, following the 4-wk diet period; Pre, before beginning the 4-wk diet period; QUICKI, quantitative insulin sensitivity check index; TFR, transferrin receptors.
[2] *P* value calculated according to PROC MIXED ANOVA for crossover design with 2 periods comparing the changes during the 4-wk diet periods (post − pre) between the lean seafood and nonseafood diet periods. The Bonferroni correction has been performed (as described in Methods).

lean seafood diet period ($P = 0.05$) (Table 1, **Supplemental Figure 4**A–D). However, we observed a strong change during the diet periods in the postprandial TG/HDL-cholesterol ratio ($P = 0.002$), which was higher after the nonseafood diet period than after the lean-seafood diet period (Figure 1E).

Discussion

Previously we reported that 4 wk of lean seafood intake, compared with nonseafood intake, reduced postprandial concentrations of TG and VLDL particles and reduced the total-to-HDL-cholesterol ratio (23). The development of an atherogenic lipoprotein profile with high VLDLs and low HDL cholesterol levels is thought to precede the diagnosis of T2D by several years (39). Thus, in the present study we aimed to elucidate the effects of lean seafood and nonseafood intake on postprandial glucose metabolism in healthy adults.

During the dietary periods, there was a reduction in postprandial C-peptide concentration after the lean seafood diet period relative to after the nonseafood diet period. An elevated C-peptide concentration is considered an early indicator of insulin resistance (25). We observed no diet-induced changes in serum glucose concentration; hence, the ability to regulate the serum glucose level was maintained at a relative lower C-peptide concentration, suggesting improved insulin sensitivity after the lean seafood diet period. Moreover, we recently reported that the changes observed in the urinary metabolome after consumption of the lean seafood diet period indicated improved insulin sensitivity, compared with after the nonseafood diet period (40). Cod intake, relative to nonseafood intake, has previously been shown to improve insulin sensitivity in insulin-resistant subjects (17). In keeping with the protection from an elevated TG concentration and total/HDL-cholesterol ratio after the lean seafood diet period as reported by us previously (23), hepatic insulin resistance has been suggested to be a key element in the development of metabolic dyslipidemia and hepatic steatosis (41). An elevated fasting C-peptide concentration has been associated with fatty liver (42). Hence, the observed reduction in C-peptide concentration after the lean seafood diet period may reflect lower liver lipids in the participants. In rodents, consumption of lean seafood reduces hepatic lipid concentration (12, 43–45). Thus, based on our previously published data on lipoprotein profiles (23) and changes in the urinary metabolome (40) and the present findings that serum glucose concentration was maintained at a lower C-peptide concentration, it is suggested that lean seafood intake improved or maintained insulin sensitivity in healthy adults, relative to nonseafood intake.

Despite no change during the diet period on postprandial glucose concentration, the postprandial lactate concentration changed during the diet periods and was lower after the lean seafood than after the nonseafood diet period. Hence, the diets may have altered the intracellular glucose metabolism differently. After intake of carbohydrates, a substantial amount of the blood glucose is taken up by tissues and stored as glycogen in

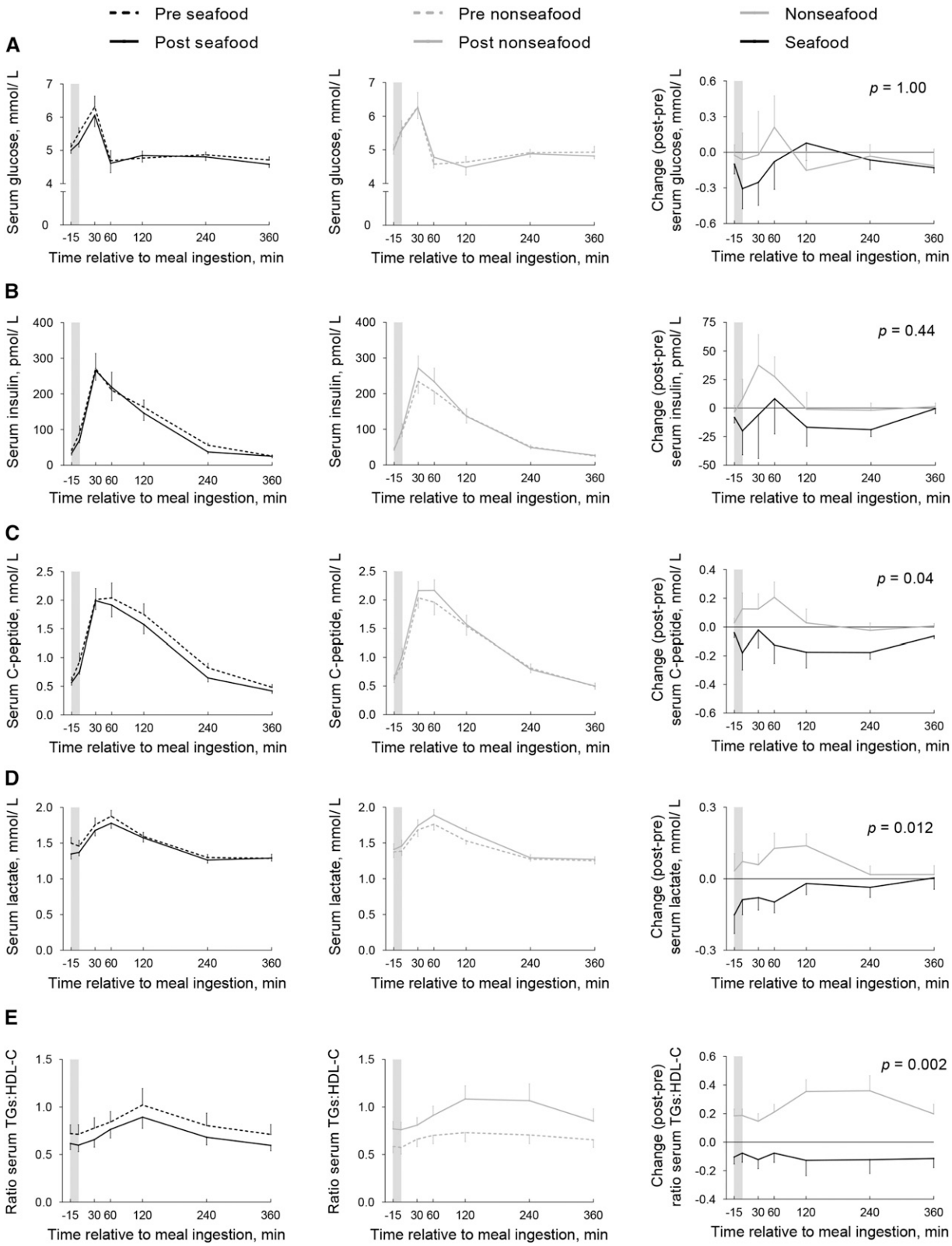

FIGURE 1 Postprandial serum glucose metabolism variables and the TG/HDL-C ratio in healthy adults before and after 4-wk periods of consuming lean seafood and nonseafood diets in a crossover design. Values are means ± SEMs, n = 20 (lean seafood) or 19 (nonseafood). Glucose (A), insulin (B), C-peptide (C), and lactate (D) concentrations and the TG/HDL-C ratio (E) were determined while fasting (−15), and immediately after (0) and at 30, 60, 120, 240, and 360 min after test meal intake. The shaded areas indicate time of test-meal ingestion (15 min). P values were assessed by repeated measures ANOVA for crossover design with 2 periods comparing the changes during the diet periods (post − pre measures) for the lean seafood and nonseafood diet periods. The Bonferroni correction has been performed (as described in Methods). HDL-C, HDL cholesterol; Post, following the 4-wk diet period; Pre, before beginning the 4-wk diet period.

insulin-sensitive subjects, whereas this insulin-stimulated whole-body glucose disposal (i.e., glycogen synthesis) is impaired in insulin-resistant subjects (24, 46, 47). When the storage capacity of glucose as glycogen is reduced, a higher proportion of ingested carbohydrates enter glycolysis and thereby increase postprandial lactate levels (47, 48). Our observation that the lean seafood diet period, relative to the nonseafood diet period, reduced postprandial lactate but not postprandial glucose may thus relate to a higher capacity to store glucose as glycogen. Furthermore, because elevated serum lactate has been suggested to be a marker of deteriorating glucose tolerance later in life (49) and to predict incident diabetes (50), our findings support the notion that lean seafood intake may preserve insulin sensitivity in healthy adults.

Reduced insulin sensitivity may have contributed to the elevated TG/HDL-cholesterol ratio that evolved in the subjects after the nonseafood diet period. When a higher proportion of the ingested carbohydrates was diverted away from glycogen storage and into hepatic de novo lipogenesis, a 60% increase in serum TG and ~20% reduction in HDL cholesterol concentrations were observed in young, lean, insulin-resistant subjects (24). Importantly, the dyslipidemia induced by insulin resistance as reported by Petersen et al. (24) appeared independent of systemic inflammation, changes in adiponectin concentration, and intra-abdominal obesity. Thus, despite no diet effect on changes in fat mass or adiponectin concentrations, it is possible that the observed different change in postprandial lactate concentration was an early indicator of diet-induced altered insulin sensitivity, contributing to the observed difference in TG/HDL-cholesterol ratio.

The present study was not designed to identify mechanisms underlying the observed diet effects. However, it is possible that the elevation in postprandial C-peptide and lactate concentrations and that in the TG/HDL-cholesterol ratio after the nonseafood diet period were caused by the intake of red meat, previously associated with increased fasting glucose and insulin concentrations (51) and increased risk of T2D development (11, 52, 53). A low serum glycine concentration, together with a high level of ferritin and altered hepatic derived lipid metabolite concentrations, has been associated with red meat intake and T2D risk (54). In the present study, we observed no diet effect on serum ferritin concentration, but the dietary glycine content was higher in the lean seafood than in the nonseafood diet, 4.7% compared with 4.2% of total amino acids, respectively (23). Also, dietary taurine content was higher in the lean seafood than in the nonseafood diet: 0.3% compared with 0.1% of total amino acids, respectively (23). Human studies on taurine supplementation with regard to insulin sensitivity are this far inconclusive, showing either improved (55) or no effect (56). Thus, further studies are needed to elucidate whether amino acid composition or other dietary components were underlying factors affecting the observed differences in the present study.

The limitations of our study relate to the design of the study. First, the practical issues of preparing, with all ingredients weighed, and serving 2 meals/day for 28 d over 2 diet periods, limited the number of subjects that could be included. Next, the study was demanding to the participants, reflected by the 26% dropout (7 of 27). This was, however, accounted for in the power analysis before the study. In addition, the validity of the ratio between TG/HDL cholesterol as a proxy for insulin sensitivity is acceptable for white subjects, but not for African Americans (29–31). Moreover, because of 1 omelet meal/wk in the nonseafood menu, this diet provided a higher weekly cholesterol intake. Previous studies have reported that an intake of ~1 egg/d, which is considerably higher than in the present study, was (57) or was not (58) associated with increased risk of incident T2D. Furthermore, in order to elucidate the potential of lean seafood or nonseafood diets to modulate postprandial glucose metabolism in 4 wk, we gave the subjects 60% of total dietary protein from lean seafood or nonseafood sources, which for most individuals is higher than a regular intake. Finally, a mean of 3.3 g (3.5 mL) of cod liver oil was added to each dinner meal of the nonseafood diet (10,900 kJ/d) and in addition to the marine n-3, the daily vitamin contribution from the cod liver oil supplement was 175 μg of vitamin A, 7 μg of vitamin D, and 7 mg of vitamin E. We cannot rule out that these issues might have influenced the results or limited the generalizability of the trial findings to a broader population.

We conclude that in healthy adults, a 4-wk diet period with 60% of the dietary protein from lean seafood reduced postprandial C-peptide and lactate concentrations and the TG/HDL-cholesterol ratio, compared with a 4-wk diet period with 60% of the dietary proteins from nonseafood sources. The diets did not alter serum insulin and glucose concentrations; therefore, these data show that postprandial different lactate concentrations occurred at normal glucose and insulin concentrations in otherwise healthy adults. Based on the data from the present study, we hypothesize that the observed elevated lactate concentration reflected a metabolic shift with increased glycolysis that provided substrates for increased hepatic lipogenesis, explaining the elevated TG/HDL-cholesterol ratio after the nonseafood intervention. We conclude that the dietary protein source regulates postprandial concentrations of C-peptide and lactate and the ratio of TG/HDL cholesterol in healthy adults, which may affect the long-term development of insulin resistance, type 2 diabetes, and cardiovascular disease.

Acknowledgments

EKA, IEG, CL, HJ, and BL designed the study; EKA, IEG, CL, ØE, AH, GM, HJ, and BL conducted the research; MP performed statistical analysis under the supervision of HJ; EKA, IEG, CL, ØE, MP, AH, GM, LM, HJ, and BL contributed to the data interpretation; LM, HJ, and BL had primary responsibility for final content. All authors wrote the paper, and read and approved the final manuscript.

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