Aquaculture Nutrition

doi: 10.1111/j.1365-2095.2009.00693.x

*Aquaculture Nutrition* **2010 16;** 536–543

# The effect of hepatic passage on postprandial plasma lipid profile of rainbow trout (*Oncorhynchus mykiss*) after a single meal

E.J. ELIASON[1], B. DJORDJEVIC[2], S. TRATTNER[3], J. PICKOVA[3], A. KARLSSON[2,4], A.P. FARRELL[1,5] & A.K. KIESSLING[2,4]

[1] *Department of Zoology, University of British Columbia, Vancouver, BC, Canada;* [2] *Department of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Aas, Norway;* [3] *Department of Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden;* [4] *Aquaculture Protein Centre (APC), Centre of Excellence, Aas, Norway;* [5] *Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada*

## Abstract

For the first time, pre- and post-hepatic plasma lipid profiles were monitored following a single meal in a free-swimming, non-anaesthetized fish. Rainbow trout (*Oncorhynchus mykiss;* 700–1500 g; 10 °C) were equipped with cannulae in the dorsal aorta (DA) and hepatic portal vein (HPV). Simultaneous blood samples, taken from both cannulae at 0, 3, 6, 12, 24 and 48 h postprandial, revealed the time course of the plasma lipid profiles following a single meal (1% of body mass). Primarily monounsaturated fatty acids with the exception of 18:1*n* − 9, increased significantly from baseline by 12 h postprandial without greatly affecting total plasma lipid concentrations. Total plasma lipids then showed a small peak at 24 h postprandial, coinciding with a peak in triacylglycerols. We conclude that assimilation of lipids from the digest into the plasma is slower than reported for proteins and carbohydrates in the same species. Furthermore, as there were no significant differences between the HPV and DA, no measurable effect of hepatic passage on plasma lipid levels was resolved. Therefore, we also conclude that, in contrast to that in higher vertebrates, hepatic passage does not seem to have a major role in rainbow trout for modulating the postprandial plasma profile of lipids.

**KEY WORDS**: dorsal aorta cannulation, fatty acid metabolism, fatty acids, hepatic portal vein cannulation, lipid metabolism, liver metabolism

Received 21 January 2009, accepted 12 May 2009

*Correspondence: Prof. Anders Kiessling, Aquaculture Protein Centre (APC), P. O. Box 5003, N-1432 Aas, Norway. E-mail: anders.kiessling@umb.no*

## Introduction

When vertebrates digest a meal, nutrients are transported across the gut wall and into either the blood or the lymph. In mammals, it is well recognized that the digestion of lipids produces fatty acids (FA), monoglycerols and glycerol, which then diffuse into absorptive cells of the gut. The triacylglycerols (TAG) are reassembled in the smooth endoplasmic reticulum, combined with phospholipids (PL), apo-lipoproteins and cholesterol to form the chylomicrons that are subsequently found in the circulation. In humans, around 80% of these chylomicrons enter the lymphatic system for distribution, with the remainder entering the hepatic portal vein (HPV). In contrast, details of lipid absorption and transport are poorly understood in fish, because their lymphatic system is not as advanced as in mammals (Steffensen & Lomholt 1992). In addition, there is the challenge of studying small aquatic animals (Sargent *et al.* 2002) and particularly the need to sample blood from the HPV. In a review by Babin & Vernier (1989), plasma is suggested as the main transporter of lipids, e.g. plasma transports three times more lipid in fed rainbow trout than in fed rats. Thus, while dietary lipids are certainly efficiently absorbed in fishes and are probably absorbed primarily as free FAs (Kayama & Iijima 1976), possibly as chylomicrons (Sheridan *et al.* 1985), the route of lipid transport to the liver and beyond remains unclear (Sheridan 1988). Both, the HPV and the lymphatics, remain as candidate routes for uptake from the intestinal epithelium (Rust 2002).

Past studies on lipid absorption and metabolism have benefited from *in vitro* studies (Weber *et al.* 2002), but *in vivo* using tracers (Haman & Weber 1996; Haman *et al.* 1997) have suffered from the obvious shortcomings of serially

slaughtering fish postprandial (reviewed by Carter *et al*. 2001). The advent of a reliable HPV cannulation technique for free-swimming Atlantic salmon, *Salmo salar* (Eliason *et al*. 2007), provides a more sensitive method to follow nutrient uptake in fish. Furthermore, comparison of plasma concentrations of amino acids in blood sampled simultaneously from the HPV and the dorsal aorta (DA) (Soivio *et al*. 1975) has provided evidence of postprandial hepatic modification of free amino acids in rainbow trout, *Oncorhynchus mykiss* (Karlsson *et al*. 2006). This observation then raises the possibility that FAs too could undergo hepatic modification immediately after absorption and transport by the HPV.

The primary objective of the present study was to utilize the HPV and DA cannulation techniques used in earlier studies to simultaneously characterize the postprandial plasma lipid profile and thereby examine plasma lipid modifications during their initial hepatic passage in free-swimming, non-anaesthetized rainbow trout following a single meal.

## Materials and methods

### *Experimental conditions*

The experimental conditions are detailed elsewhere (Karlsson *et al*. 2006). Briefly, rainbow trout (700–1500 g, $n = 12$, average 1100 g) were held at the Norwegian University for Life Sciences (UMB, Ås, Norway) in a 1000-L indoor tank with fully aerated, recirculating well-water at 10.0 ± 0.1 °C. The fish were fed commercial trout chow (Ewos, Opalsa Silva 30A, Norway) 5 days a week to satiation, and starved for 48 h prior to DA surgery and an additional recovery period of 48 h before HPV surgery. Anaesthetized fish (0.1 g $L^{-1}$ buffered MS-222; Norwegian Medical Depot, Oslo, Norway) were placed on a surgery table where their gills were continually irrigated with a chilled, aerated buffered MS-222 solution (0.05 g $L^{-1}$). The DA (Soivio *et al*. 1975; as modified by Kiessling *et al*. 1995, 2003) and then the HPV (Eliason *et al*. 2007) were cannulated. Fish recovered for 24 h under continuous illumination with daylight fluorescent tubes in individual 0.5 $m^2$ experimental compartments, which received aerated, recirculating well-water (10 ± 0.1 °C, 2 L $min^{-1}$). The compartments were fitted with a 200 mm × 400 mm cover, 50 mm above the water surface that functioned as a shelter. Visually, all fish were in good health during the experiment and the surgical incisions were free from infection. The findings that leucocrit remained stable and that both alanine aminotransferase and aspartate aminotransferase varied little indicated that these procedures did not cause major tissue damage (data and procedures reported detailed in Karlsson *et al*. 2006). In addition, previous studies using these procedures have shown that plasma cortisol levels subside to a routine level after the 24-h recovery period (Eliason *et al*. 2007) and salmonids digest a single meal given by gavage (data presented in Karlsson *et al*. 2006). Therefore, blood was sampled for control conditions after a 24-h recovery (see below) and then fish were re-anaesthetized (as described above) and given a single meal of 1% of their body mass by gavage. The meal, which followed at least 5 days without feeding, resulted in an average lipid intake of 3.5 g per fish (lipid content of diet 315 g $kg^{-1}$). Fish were returned into their individual compartment, where postprandial lipid uptake was followed. Pellet regurgitation was negligible in all cases based on visual monitoring. Blood sampling (400–500 μL) from both cannulae occurred at 3, 6, 12, 24 and 48 h postprandial, as well as immediately prior to feeding (control = 0 h). Each blood sample was centrifuged at 500 ***g*** for 5 min and the plasma was stored immediately at −20 °C before being transferred within hours to −80 °C until analysis. An 80-μL plasma aliquot was used for lipid analysis and the remainder was used to measure plasma amino acid profiles (Karlsson *et al*. 2006).

This study was conducted as a part of a larger experiment that targeted effects of protein source on plasma amino acid profile by partial substitution of fish meal by corn gluten meal. A detailed description of the diets is given in Karlsson *et al*. (2006). Proximate composition of the diets (fish meal/corn gluten meal) given as percentage of dry matter (DM) was as follows; DM 95/95, protein ($N \times 6.25$) 36.5/37.0, fat 30.0/30.0, starch 8.0/8.3, ash 8/6, non-starch carbohydrates 10.0/10.4. Because the diets were isocaloric, using the same lipid source (fish oil), only one FA (18:2$n$ − 6) differed between diets (1.59 and 3.9% of identified FAs, respectively). All other FAs differed by less than the analytic error between the two diets. Therefore, Table 1 reports the average of both diets. Furthermore, the dietary difference in 18:2$n$ − 6 resulted in a minor difference in plasma concentrations (1.61 ± 0.05 versus 1.95 ± 0.04% of identified FAs, respectively). Furthermore, because no significant differences in ($P > 0.05$) were observed between the DA and HPV when fish were grouped according to type of diet and there was no interaction between diet and sampling time, we concluded that data from the two diets could be pooled in the statistical evaluation.

### *Fatty acid and lipid class analysis*

The feed pellets were ground through a 0.5-mm screen and analyzed for DM (EC 1971b), ash (EC 1971a), crude protein (EC 1993), crude fat (EC 1998), starch (McCleary *et al*. 1994),

**Table 1** Dietary fatty acid (FA) composition

| Dietary FA (%) | Diet |
|---|---|
| 14:0 | 6.14 |
| 16:0 | 12.2 |
| 16:1*n* − 7 | 6.16 |
| 18:0 | 1.40 |
| 18:1*n* − 11 | 1.10 |
| 18:1*n* − 9 | 9.36 |
| 18:1*n* − 7 | 2.36 |
| 18:2*n* − 6 | 2.51 |
| 18:3*n* − 3 | 0.89 |
| 20:1*n* − 11 | 3.09 |
| 20:1*n* − 9 | 11.3 |
| 20:1*n* − 7 | 0.43 |
| 20:4*n* − 6 | 0.30 |
| 20:4*n* − 3 | 1.2 |
| 20:5*n* − 3 | 7.52 |
| 22:1*n* − 11 | 16.0 |
| 22:1*n* − 9 | 1.41 |
| 22:1*n* − 7 | 0.48 |
| 22:5*n* − 3 | 0.62 |
| 22:6*n* − 3 | 8.06 |
| 24:0 | 0.33 |
| 24:1*n* − 9 | 0.73 |

The FA values are given as percentage of total identified FA. FA that do not constitute >0.3% of the total are not included in the table.

non-starch polysaccharides (Lee *et al*. 1992), amino acid composition (EC 1998) and FA composition (see below for details). Lipid extraction was performed on duplicate 2.5-g feed samples (Folch *et al*. 1957) and duplicate 40-μL plasma samples (Hara & Radin 1978). The lipid content was measured gravimetrically. Then lipid classes were analyzed (Olsen & Henderson 1989) in triplicate on a TLC plate with a CAMAG TLC sampler 4 (Camag, Switzerland). Lipid classes were compared with an external standard (TLC 18-4A, Nu-Chek Prep, Elysian, MN, USA). Extracted plasma lipids and lipids from feed samples were methylated (Appelqvist 1968; Hoshi *et al*. 1973; Thomassen *et al*. 1979) prior to gas chromatography analysis (CP9001, Chrompack, Middelburg, the Netherlands) using a CP9050 auto sampler to inject samples (1 μL). Fatty acid profiles and retention times were compared as a standard (GLC-68 A, Nu-Chek Prep, Inc, Elysian, MN, USA). Peak areas were integrated using a Maestro 2 version 2.4 Integrator (Chrompack, Middelburg, the Netherlands).

### *Statistical analyses*

Data were analyzed using the Statistical Analysis System (SAS, version 8.02 for Windows). Sample time, diet and blood vessel were tested by a main factorial model (GLM procedure for unbalanced data). Fish was included as a discrete variable. Groups were compared by the *ad hoc* variance test (*F*-test) using the least-squares means (ls mean) procedure when significant effects were found in the main model. *P* values $<0.05$ were considered to be statistically significant. All data were tested for normality by a normal probability plot (proc univariate plot).

## Results

The postprandial changes in total plasma lipid and lipid classes are shown in Table 2. At no time did the total plasma lipid concentrations differ significantly ($P > 0.05$) between the DA and HPV samples and the profiles were the same for the DA and HPV samples.

Total plasma lipids increased between 12- and 24-h postprandial and then decreased at 48-h postprandial. Similarly, the plasma lipid classes [TAG, free fatty acids (FFA), cholesterol (CL), unsaturated FA-cholesterol esters (U), PL and saturated FA-cholesterol esters (S)] were not significantly different between the DA and HPV at any time point (Table 2), except for a few minor temporal variations for some classes. In particular, the only lipid class that demonstrated a significant difference between the two sample sites was the minor difference in PL at 6-h postprandial. Sample site was therefore kept as a covariate in the statistical model when comparing the ls-mean data for the sample times.

Both S and U increased immediately postprandial (3 h), reaching a peak at 6-h postprandial and returning to control levels at 24-h postprandial (Table 2; Fig. 1). In contrast, TAG decreased initially and peaked after 24-h postprandial (Table 2; Fig. 1).

The individual plasma fatty acids (PFA) are grouped according to three distinct postprandial patterns (Table 3). $PFA_1$ was used to denote a plasma FA that increased postprandial (Fig. 2a). The $PFA_1$ grouping was primarily short-chain saturated and monounsaturated fatty acids, with the exception of 18:3 and 18:4*n* − 3 (Table 3). $PFA_1$ remained steady for the first 6-h postprandial and then increased to 250% of the control level (Fig. 2a). $PFA_2$ was used to denote a plasma FA that decreased postprandial (Table 3). The $PFA_2$ group was predominately long-chain saturated or highly unsaturated fatty acids and remained steady over the first 6-h postprandial but decreased significantly by 12-h postprandial (Fig. 2b). $PFA_3$ was used to denote the remaining plasma FA that were unchanged over time (Table 3).

## Discussion

This study presents the first simultaneous, postprandial measurements of plasma lipids in pre- and posthepatic blood

**Table 2** Postprandial changes in total concentration of plasma lipids (TL) and relative lipid class composition (%) of phospholipids (PL), triacylglyceroles (TAG), free fatty acids (FFA), cholesterol (CL), unsaturated cholesterol esters (U) and saturated cholesterol esters (S), collected simultaneously from the dorsal aorta (DA) and hepatic portal vein (HPV) in free-swimming rainbow trout after a single meal

| | Vessel/h | 0 | 3 | 6 | 12 | 24 | 48 |
|---|---|---|---|---|---|---|---|
| TL (μg μL plasma$^{-1}$) | DA | 11.7 ± 0.74a | 11.8 ± 0.86a | 10.6 ± 0.90a | 12.3 ± 1.01a | 12.0 ± 0.90a | 8.86 ± 0.95b |
| | HPV | 11.1 ± 0.86ac | 12.6 ± 0.90ab | 10.6 ± 0.89ac | 12.3 ± 0.89ab | 13.8 ± 0.99b | 8.84 ± 0.99c |
| PL (%) | DA | 23.2 ± 0.61a | 22.0 ± 0.71a | 23.8 ± 0.74a* | 23.0 ± 0.83a | 22.4 ± 0.74a | 19.5 ± 0.78b |
| | HPV | 24.4 ± 0.88a | 22.4 ± 0.92ab | 21.6 ± 0.92b | 22.9 ± 0.92ab | 24.2 ± 1.00ac | 21.8 ± 1.02bc |
| TAG (%) | DA | 22.5 ± 1.44a | 18.7 ± 1.68b | 19.3 ± 1.76ab | 23.6 ± 1.97ac | 27.0 ± 1.76c | 17.5 ± 1.85b |
| | HPV | 23.4 ± 1.53ac | 19.9 ± 1.60ab | 17.7 ± 1.60b | 22.4 ± 1.60ac | 25.6 ± 1.68c | 20.8 ± 1.77ab |
| FFA (%) | DA | 6.34 ± 0.50a | 4.83 ± 0.59b | 3.80 ± 0.62b | 4.25 ± 0.69b | 4.74 ± 0.62b | 5.43 ± 0.69ab |
| | HPV | 6.40 ± 0.71a | 4.42 ± 0.74bc | 4.63 ± 0.74ab | 4.09 ± 0.74b | 5.23 ± 0.78ab | 6.22 ± 0.82ac |
| CL (%) | DA | 11.4 ± 0.42ab | 11.1 ± 0.50ab | 11.8 ± 0.52ab | 10.7 ± 0.58b | 10.9 ± 0.52b | 12.6 ± 0.58a |
| | HPV | 11.8 ± 0.55a | 11.6 ± 0.57ab | 10.8 ± 0.57ab | 10.2 ± 0.57b | 11.3 ± 0.60ab | 12.5 ± 0.63a |
| Cholesterol esters U (%) | DA | 18.9 ± 1.09a | 22.0 ± 1.28b | 21.1 ± 1.34ab | 19.2 ± 1.50ab | 17.9 ± 1.34a | 21.1 ± 1.41ab |
| | HPV | 17.8 ± 1.13ac | 20.7 ± 1.18ab | 22.4 ± 1.18b | 20.2 ± 1.18ab | 17.1 ± 1.24c | 19.9 ± 1.30abc |
| Cholesterol esters S (%) | DA | 17.7 ± 1.41a | 21.4 ± 1.64bc | 20.2 ± 1.73ac | 19.3 ± 1.93ac | 17.1 ± 1.73a | 19.4 ± 1.82ac |
| | HPV | 16.1 ± 1.65a | 21.0 ± 1.72b | 22.8 ± 1.72b | 20.7 ± 1.72b | 16.6 ± 1.80a | 18.8 ± 1.90ab |

Values are given as ls-mean ± SEM including fish as discrete variables in the statistical model. Values from the same vessel followed by the same letter do not differ significantly ($P > 0.05$).
*$P < 0.05$ statistically significant difference between the DA and HPV values at a given sample time.

of a free-swimming, non-anaesthetized fish. Our results clearly demonstrate that there are fundamental differences in postprandial uptake and modification of lipid classes and individual FAs compared with amino acids in rainbow trout. While the postprandial changes in total plasma lipids were quantitatively minor and with no significant differences between the HPV and DA lipid levels, we found evidence that amino acid uptake was well underway after 3-h and completed between 24-h and 48-h postprandial with significant differences in plasma amino acid levels between the HPV and DA (Karlsson *et al*. 2006).

The lack of a simple quantitative relationship between plasma and dietary levels for individual FA is in contrast to the well-established relationship between dietary profile and deposited lipids in salmonids (Kiessling *et al*. 2001). For example, 20:1 and 22:1 represent almost 30% of the dietary FA, but less than 5% of plasma FA. Similarly, 22:6*n* − 3 makes up less than 10% of the dietary FA, but close to 30% of plasma FA. In contrast, good quantitative relationships exist between dietary and plasma 18:1 and 18:2 FA. The discrepancy between dietary and circulating lipids may indicate the multifaceted metabolic interactions between lipid accumulation, mobilization and depletion. In humans, the complexity of plasma responses to dietary intervention is linked to the individual genetic background with several candidate genes as promising markers of individual dietary responsiveness (Ordovas 2001). The recent findings show that stored lipids are not only a fuel reservoir (Tomas *et al*. 2004), but also an endocrine organ (Yu & Ginsberg 2005) that communicates with the rest of the body and keeps body homeostasis, thus influencing the circulating plasma lipid levels as well. Furthermore, lipids are known to influence gene expression and prostaglandin synthesis (Zheng *et al*. 2005; Bell *et al*. 1995).

The present study revealed distinct postprandial patterns among lipid classes that correspond with earlier studies. U and S increased significantly from baseline by 3-h postprandial, whereas TAG decreased between 3-h and 6-h postprandial, but increased at 24-h postprandial. However, PL, FFA and CL varied little postprandially. These results correspond well with a study in sea bass (*Dicentrarchus labrax)*, where PFA reached a peak at 12-h postprandial and CL remained constant (Santulli *et al*. 1988).

The relatively small postprandial increases in HPV plasma lipid levels for most of the lipid classes and the lack of significant differences in individual PFA between the DA and HPV sample sites can be interpreted in three different ways. First, circulating lipid levels and composition may be tightly controlled by regulating the uptake or release of lipids from adipose and hepatic tissue in order to balance the absorption from a meal. Such regulation would only be possible if the uptake is very slow. The marked quantitative differences in plasma FA and dietary FA profiles, exemplified by 22:6*n* − 3, indicate such interpretation, especially if one considers that adipose tissue of rainbow trout reflects the FA composition of diet (Kiessling *et al*. 2001). Second, the data could imply a prominent role for uptake via the lymphatic/secondary circulatory system, and perhaps the HPV is a secondary route.

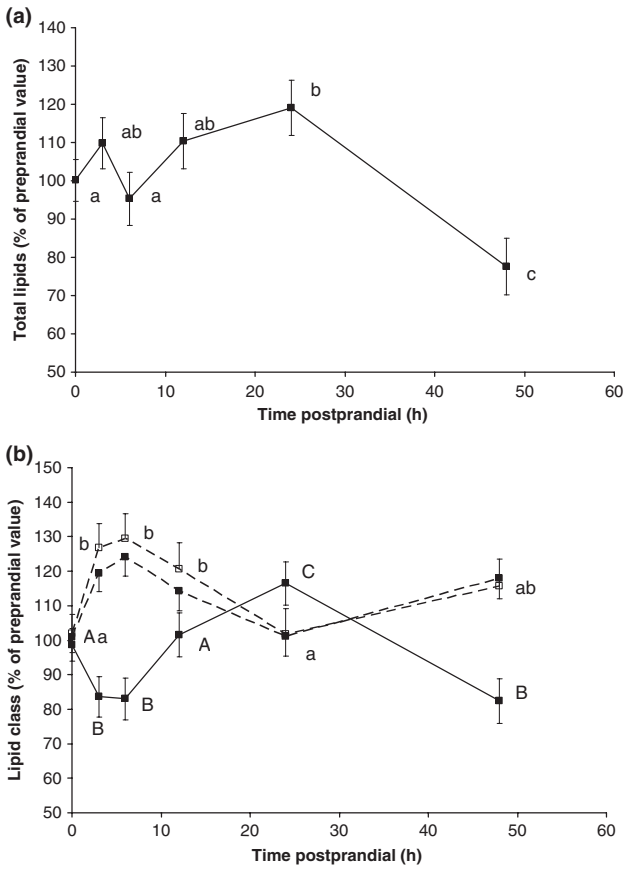


**Figure 1** Postprandial changes (the control sample is set to 100%) in total plasma lipids (a) and plasma lipid classes (b) (triacylglycerols, TAG, black squares-solid line and saturated cholesterol esters, S, black squares-dotted line and unsaturated cholesterol esters, U, white squares-dotted line). Values are given as ls-mean ± SEM per sample time, including time, vessel and individual fish as discrete variables of the model. Values followed by the same letter (upper and lower case letters are used for TAG and cholesterol esters, respectively) do not differ significantly ($P > 0.05$). For simplicity and because S and U do not differ, lower case letters in the figures are indicative of both S and U.

Such an interpretation would imply that the intestine rather than the liver would be the metabolically active tissue in rainbow trout. Sire *et al.* (1981) provided ultrastructural evidence that re-esterification of the FAs takes place in rainbow trout intestinal cells and, by following radiolabelled FAs, concluded that postprandially, lipids are transported (slowly) via the lymph because radioactivity remained low for 2 h in the liver and for 6 h in the plasma. However, Rogie & Skinner (1985) observed that liver was more active than the intestine, concluding that in fish, liver is the primary organ of lipoprotein biogenesis. In addition, both Babin & Vernier (1989) and Lie *et al.* (1993) referred to plasma as the main lipid transport system. Thirdly, the present data could indicate that lipid absorption and metabolism in rainbow trout is too slow to yield pre- and post-hepatic differences, and therefore the plasma reflects a steady state. Indeed, Robinson & Mead (1973) found that plasma lipid composition remained surprisingly unchanged between 8-h and 14-h postprandial.

The marked 48-h postprandial decrease in plasma TL (Fig. 1a) is surprising because intuitively one would expect the 48-h sample to be similar to the control one. However, the control sample likely represents a starvation state because it followed at least 5 days without feeding. The 48-h sample, in contrast, likely represents a postabsorptive state, i.e. it might indicate a phase preceding the one where endogenous fuels maintain plasma lipid levels in parity with the initial drop in blood glucose of fish entering a period of starvation (Suarez & Mommsen 1987). The parallel reduction in plasma TAG at 48-h postprandial supports such an assumption as it may reflect removal of TAG in response to an energy need of metabolically active tissues.

The majority of FA transport involves the incorporation of FA into one lipid class or another. Our postprandial patterns for individual plasma FA suggest different metabolic fates for different FA. The increase in $PFA_1$ coincides with a general increase in cholesterol esters and a decrease in TAG indicates that the primary phase of absorption via the HPV route involves short-chain and saturated FA incorporated into cholesterol esters and not TAG. This phase does not involve a significant change in total plasma lipid level and the relative increase of one FA or a lipid class needs to be balanced by a decrease in another, i.e. the relative measurements used in the present study, because of limitations in sample volume of plasma, are in this situation indicative also of absolute changes. Indeed, the increase in cholesterol esters is in part balanced by a general decrease in all other lipid classes (Table 2) and TAG in particular (Fig. 1). It is also noteworthy that the increase in $PFA_1$ is balanced by an apparent decrease in $PFA_2$. The primary uptake phase is then changed into a quantitatively more pronounced phase involving a small increase in plasma TL and a major relative increase in TAG, possibly indicative of appearance of chylomicrons by postprandial translocation via the lymphatics. This increase in TAG is mainly balanced by a decrease in cholesterol ester. However, because of the increase in total lipids, this relative decrease of cholesterol esters may in fact only be apparent and the amount of cholesterol esters could remain steady. Thus, the decrease in $PFA_2$ of more long-chain and unsaturated FA in this phase could simply reflect this infusion of TAG, resulting in a higher relative amount of more saturated, short-chain FA typical of TAG and a steady content of $PFA_2$.

**Table 3** Postprandial changes in total plasma fatty acid levels (PFA, percentage of total analyzed) collected simultaneously from the dorsal aorta (DA) and hepatic portal vein (HPV) in free-swimming rainbow trout after a single meal

| | (%) | Vessel/h | 0 | 3 | 6 | 12 | 24 | 48 |
|---|---|---|---|---|---|---|---|---|
| $PFA_1$ | 14:0 | DA | 1.55 ± 0.10a | 1.63 ± 0.12a | 1.45 ± 0.12a | 1.88 ± 0.15a | 2.21 ± 0.12b | 1.73 ± 0.13a |
| | | HPV | 1.40 ± 0.14a | 1.29 ± 0.14a | 1.52 ± 0.14a | 1.90 ± 0.14bc | 2.25 ± 0.16c | 1.67 ± 0.16ab |
| | 16:1 | DA | 1.92 ± 0.16a | 2.01 ± 0.18a | 2.01 ± 0.19a | 2.85 ± 0.21b | 3.06 ± 0.19b | 2.20 ± 0.20a |
| | | HPV | 1.83 ± 0.17a | 1.69 ± 0.17a | 1.97 ± 0.17ab | 2.47 ± 0.17c | 2.68 ± 0.19c | 2.37 ± 0.19bc |
| | 18:1*n* − 7 | DA | 1.55 ± 0.05a | 1.56 ± 0.06a | 1.65 ± 0.06ab | 1.71 ± 0.07ab | 1.74 ± 0.06b | 1.64 ± 0.06ab |
| | | HPV | 1.57 ± 0.06a | 1.57 ± 0.06a | 1.65 ± 0.06a | 1.69 ± 0.06a | 1.71 ± 0.07a | 1.65 ± 0.07a |
| | 18:3*n* − 3 | DA | 0.34 ± 0.02ab | 0.28 ± 0.03ab | 0.29 ± 0.03ab | 0.39 ± 0.03bc | 0.41 ± 0.03c | 0.34 ± 0.03ab |
| | | HPV | 0.31 ± 0.03a | 0.33 ± 0.03a | 0.31 ± 0.03a | 0.38 ± 0.03ab | 0.42 ± 0.03b | 0.33 ± 0.03ab |
| | 18:4*n* − 3 | DA | 0.27 ± 0.08a | 0.26 ± 0.08a | 0.41 ± 0.08ac | 0.79 ± 0.08b | 0.89 ± 0.07b | 0.55 ± 0.08c |
| | | HPV | 0.37 ± 0.09a | 0.28 ± 0.09a | 0.40 ± 0.09ac | 0.71 ± 0.08b | 0.81 ± 0.09b | 0.61 ± 0.09bc |
| | 20:1 | DA | 1.53 ± 0.18a | 1.50 ± 0.20a | 1.85 ± 0.21a | 2.98 ± 0.24b | 3.77 ± 0.21c | 2.85 ± 0.22b |
| | | HPV | 1.61 ± 0.26a | 1.54 ± 0.27a | 1.94 ± 0.27a | 3.15 ± 0.28b | 3.59 ± 0.30b | 3.09 ± 0.30b |
| | 22:1 | DA | 0.44 ± 0.07ab | 0.37 ± 0.09ab | 0.32 ± 0.09a | 0.42 ± 0.10ab | 0.43 ± 0.09ab | 0.63 ± 0.10b |
| | | HPV | 0.38 ± 0.09ab | 0.33 ± 0.09a | 0.30 ± 0.09a | 0.47 ± 0.09ab | 0.49 ± 0.09ab | 0.34 ± 0.10a |
| $PFA_2$ | 18:0 | DA | 4.89 ± 0.19ab | 5.03 ± 0.22ab | 5.15 ± 0.23a | 4.40 ± 0.26bc | 3.95 ± 0.23c | 4.30 ± 0.24c |
| | | HPV | 5.01 ± 0.20a | 4.88 ± 0.21a | 4.79 ± 0.21ab | 4.33 ± 0.21b | 3.83 ± 0.24b | 4.10 ± 0.23b |
| | 20:4*n* − 6 | DA | 1.37 ± 0.04ac | 1.53 ± 0.05b | 1.47 ± 0.05ab | 1.24 ± 0.06c | 1.10 ± 0.05d | 1.26 ± 0.06c |
| | | HPV | 1.45 ± 0.05a | 1.41 ± 0.06ab | 1.49 ± 0.06a | 1.29 ± 0.06bd | 1.06 ± 0.07c | 1.25 ± 0.06d |
| | 20:3*n* − 3 | DA | 0.76 ± 0.05a | 0.69 ± 0.06ab | 0.68 ± 0.06ab | 0.53 ± 0.07bc | 0.50 ± 0.06c | 0.66 ± 0.07ab |
| | | HPV | 0.76 ± 0.06a | 0.70 ± 0.06a | 0.78 ± 0.06a | 0.63 ± 0.06ab | 0.51 ± 0.07b | 0.60 ± 0.07ab |
| | 20:5*n* − 3 | DA | 10.7 ± 0.21ab | 11.1 ± 0.22ab | 10.5 ± 0.23bc | 10.2 ± 0.26c | 10.0 ± 0.23c | 10.9 ± 0.25ab |
| | | HPV | 10.8 ± 0.27a | 10.5 ± 0.28a | 10.6 ± 0.28a | 10.5 ± 0.31ab | 9.70 ± 0.33b | 10.9 ± 0.31a |
| | Unknown | DA | 3.24 ± 0.26a | 3.03 ± 0.28a | 3.08 ± 0.29a | 2.61 ± 0.35ab | 2.32 ± 0.29b | 2.81 ± 0.31ab |
| | | HPV | 3.32 ± 0.29a | 3.24 ± 0.30a | 3.00 ± 0.30a | 3.06 ± 0.32ab | 2.16 ± 0.34b | 2.83 ± 0.36ab |
| | 24:0 | DA | 0.49 ± 0.01a | 0.49 ± 0.02a | 0.47 ± 0.02a | 0.42 ± 0.02bc | 0.40 ± 0.02c | 0.46 ± 0.02ab |
| | | HPV | 0.50 ± 0.02a | 0.49 ± 0.02a | 0.48 ± 0.02ab | 0.45 ± 0.02b | 0.39 ± 0.02c | 0.46 ± 0.02ab |
| | 22:5*n* − 3 | DA | 2.53 ± 0.09a | 2.52 ± 0.11a | 2.40 ± 0.11ab | 2.21 ± 0.12bc | 2.06 ± 0.11c | 2.31 ± 0.12ac |
| | | HPV | 2.53 ± 0.11a | 2.45 ± 0.12a | 2.33 ± 0.12a | 2.24 ± 0.12ab | 1.97 ± 0.13b | 2.30 ± 0.13ab |
| | 22:6*n* − 3 | DA | 30.3 ± 0.72a | 30.6 ± 0.81a | 29.9 ± 0.85a | 26.6 ± 0.95b | 25.8 ± 0.85b | 30.2 ± 0.89a |
| | | HPV | 30.9 ± 0.81a | 30.8 ± 0.85a | 29.2 ± 0.85ab | 28.2 ± 0.85bc | 26.7 ± 0.99c | 29.7 ± 0.93ab |
| $PFA_3$ | 16:0 | DA | 20.0 ± 0.69 | 19.9 ± 0.78 | 19.8 ± 0.81 | 18.6 ± 0.91 | 17.8 ± 0.81 | 17.9 ± 0.86 |
| | | HPV | 19.3 ± 0.81 | 18.7 ± 0.84 | 19.3 ± 0.84 | 18.4 ± 0.84 | 18.6 ± 0.93 | 17.5 ± 0.93 |
| | 18:1*n* − 9 | DA | 10.7 ± 0.49 | 10.4 ± 0.55 | 10.6 ± 0.58 | 10.9 ± 0.65 | 10.4 ± 0.58 | 9.9 ± 0.61 |
| | | HPV | 10.4 ± 0.56 | 10.7 ± 0.58 | 10.6 ± 0.58 | 10.3 ± 0.58 | 10.7 ± 0.68 | 9.6 ± 0.64 |
| | 18:2*n* − 6 | DA | 1.81 ± 0.11 | 1.65 ± 0.12 | 1.75 ± 0.12 | 2.01 ± 0.14 | 1.96 ± 0.12 | 1.72 ± 0.13 |
| | | HPV | 1.65 ± 0.11 | 1.66 ± 0.12 | 1.94 ± 0.11 | 1.82 ± 0.11 | 1.86 ± 0.13 | 1.61 ± 0.13 |
| | 20:2*n* − 6 | DA | 0.35 ± 0.01a | 0.33 ± 0.01ab | 0.33 ± 0.01ab | 0.31 ± 0.01bc | 0.29 ± 0.01c | 0.30 ± 0.01bc |
| | | HPV | 0.35 ± 0.01 | 0.33 ± 0.01 | 0.35 ± 0.01 | 0.32 ± 0.01 | 0.29 ± 0.02 | 0.31 ± 0.02 |
| | 20:3*n* − 6 | DA | 0.19 ± 0.01 | 0.20 ± 0.01 | 0.20 ± 0.01 | 0.19 ± 0.01 | 0.18 ± 0.01* | 0.20 ± 0.02 |
| | | HPV | 0.21 ± 0.02 | 0.19 ± 0.01 | 0.21 ± 0.02 | 0.19 ± 0.01 | 0.22 ± 0.01 | 0.19 ± 0.02 |

Values are given as ls-mean ± SEM including fish as discrete variables in the statistical model. Values from the same vessel followed by the same letter do not differ significantly ($P > 0.05$).

$PFA_1$, FA with a significant increase ($P < 0.05$) between 12 and 24 h; $PFA_2$, FA with a significant decrease ($P < 0.05$) between 12 and 24 h; $PFA_3$, FA with no significant ($P > 0.05$) postprandial changes.

*Statistically significant difference between the DA and HPV values at a given sample time.

The subscript numbers following PFA denote the grouping given for the temporal analysis presented in Fig. 2.

In conclusion, postprandial variation in total plasma lipids was relatively small, compared with previous observations for amino acids. Studies with radiolabelled FA are necessary to be able to show the origin of circulating PFA and reveal hepatic FA metabolism. The current whole-animal model acted as its own control for statistical analysis, but limited the number and volume of blood samples. Potentially in contrast with the present study, species that use their liver as the primary lipid storage tissue (e.g. cod, *Gadus morhua*) may have different lipid transport and metabolic strategies to those shown here for rainbow trout.

## Acknowledgements

This study was funded through the Norwegian Research Council, Centre for Excellence, Aquaculture Protein Centre

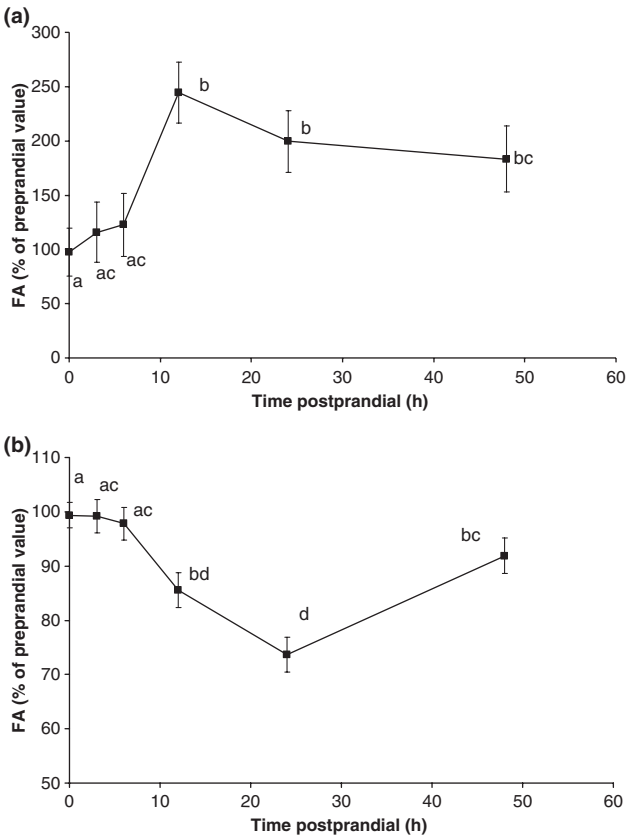


**Figure 2** Postprandial changes (the control sample is set to 100%) in groups of individual plasma fatty acids (PFA) in free-swimming rainbow trout after a single meal of 1% of body mass. Panel (a) has grouped the plasma fatty acids that show relative increase with time (denoted $PFA_1$ in Table 3), and Panel (b) has grouped the plasma fatty acids that show relative decrease with time (denoted $PFA_2$ in Table 3). Values are given as ls-mean ± SEM per sample time, including time, vessel and individual fish as discrete variables of the model. Values followed by the same letter do not differ significantly ($P > 0.05$).

(APC). Additional funds, in kind, were also provided by the Department of Animal and Aquacultural Sciences, and by the Department of Mathematics and Technology, Norwegian University of Life Sciences, Norway, to A. Karlsson. The Natural Sciences and Engineering Research Council of Canada provided funding for A.P. Farrell.

## References

Appelqvist, L. (1968) Rapid methods of lipid extractions and fatty acid methyl ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipid contaminants. *Ark. Kemi*, **28,** 551–570. .

Babin, P.J. & Vernier, J.M. (1989) Plasma lipoproteins in fish. *J. Lipid Res.*, **30,** 467–489.

Bell, J.G., Castell, J.D., Tocher, D.R., Macdonald, F.M. & Sargent, J.R. (1995) Effects of different dietary arachidonic-acid – docosahexaenoic acid ratios on phospholipid fatty-acid compositions and prostaglandin production in juvenile turbot (*Scophthalmus maximus*). *Fish Physiol. Biochem.*, **14,** 139–151.

Carter, C., Houlihan, D., Kiessling, A., Médale, F. & Jobling, M. (2001) Physiological effects of feeding. In: *Food Intake in Fish* (Houlihan, D. *et al.* eds), Vol. 1, pp. 297–331. Blackwell Science, Oxford.

EC (1971a) First commission directive 71/250/EEC of 15 June 1971 establishing Community methods of analysis for the official control of feeding stuffs. *Official J.*, **L155,** 13–37.

EC (1971b) Second commission directive 71/393/EEC of 18 November 1971 establishing Community methods of analysis for the official control of feeding stuffs. *Official J.*, **L279,** 7–18.

EC (1993) Commission Directive 93/28/EEC of 4 June 1993 amending Annex I to the third Directive 72/199/EEC establishing Community methods of analysis for the official control of feeding stuffs. *Official J.*, **L179,** 8–10.

EC (1998) Commission Directive 98/64/EC of 3 September 1998 establishing Community methods of analysis for the determination of amino-acids, crude oils and fats, and olaquindox in feeding stuffs. *Official J.*, **L257,** 14–28.

Eliason, E.J., Kiessling, A., Karlsson, A., Djordjevic, B. & Farrell, A.P. (2007) Validation of the hepatic portal vein cannulation technique using Atlantic salmon *Salmo salar* L. *J. Fish Biol.*, **71,** 290–297.

Folch, J., Lees, M. & Stanley, G.H.S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226,** 497–509.

Haman, F. & Weber, J.M. (1996) Continuous tracer infusion to measure *in vivo* metabolite turnover rates in trout. *J. Exp. Biol.*, **199,** 1157–1162.

Haman, F., Powell, M. & Weber, J.M. (1997) Reliability of continuous tracer infusion for measuring glucose turnover rate in rainbow trout. *J. Exp. Biol.*, **200,** 2557–2563.

Hara, A. & Radin, N.S. (1978) Lipid extraction of tissue with low toxicity solvent. *Anal. Biochem.*, **90,** 420–426.

Hoshi, M., Williams, M. & Kishimoto, Y. (1973) Esterification of fatty acids at room temperature by chloroform-methanolic HCl-cupric acetate. *J. Lipid Res.*, **14,** 599–601.

Karlsson, A., Eliason, E.J., Mydland, L.T., Farrell, A.P. & Kiessling, A. (2006) Postprandial changes in plasma free amino acid levels obtained simultaneously from the hepatic portal vein and the dorsal aorta in rainbow trout (*Oncorhynchus mykiss*). *J. Exp. Biol.*, **209,** 4885–4894.

Kayama, M. & Iijima, N. (1976) Studies on lipid transport mechanism in fish. *Bull. Jpn. Soc. Sci. Fish.*, **42,** 987–996.

Kiessling, A., Dosanjh, B., Higgs, D., Deacon, G. & Rowshandeli, N. (1995) Dorsal aorta cannulation: a method to monitor changes in blood levels of astaxanthin in voluntarily feeding Atlantic salmon, *Salmo salar, L. Aquac. Nutr.*, **1,** 43–50.

Kiessling, A., Pickova, J., Johansson, L., Asgard, T., Storebakken, T. & Kiessling, K.H. (2001) Changes in fatty acid composition in muscle and adipose tissue of farmed rainbow trout (*Oncorhynchus mykiss*) in relation to ration and age. *Food. Chem.*, **73,** 271–284.

Kiessling, A., Olsen, R.E. & Buttle, L. (2003) Given the same dietary carotenoid inclusion, Atlantic salmon, *Salmo salar* (*L.*) display higher blood levels of canthaxanthin than astaxanthin. *Aquac. Nutr.*, **9,** 253–261.

Lee, S.C., Prosky, L. & Devries, J.W. (1992) Determination of total, soluble, and insoluble dietary fiber in foods – enzymatic gravimetric method, MES-TRIS buffer: collaborative study. *J. AOAC Int.*, **75,** 395–416.

Lie, O., Sandvin, A. & Waagbo, R. (1993) Influence of dietary fatty acids on the lipid composition of lipoproteins in farmed Atlantic salmon (*Salmo salar*). *Fish Physiol. Biochem.*, **12,** 249–260.

McCleary, B.V., Solah, V. & Gibson, T.S. (1994) Quantitative measurement of total starch in cereal flours and products. *J. Cereal Sci.*, **20,** 51–58.

Olsen, R.E. & Henderson, R.J. (1989) The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densiometry. *J. Exp. Mar. Biol. Ecol.*, **129,** 189–197.

Ordovas, J. (2001) Gene-diet interaction and plasma lipid response to dietary intervention. *Curr Atheroscler. Rep.*, **3,** 200–208.

Robinson, J.S. & Mead, J.F. (1973) Lipid absorption and deposition in rainbow trout (*Salmo gairdnerii*). *Can. J. Biochem.*, **51,** 1050–1058.

Rogie, A. & Skinner, E.R. (1985) The roles of the intestine and liver in the biosynthesis of plasma lipoproteins in the rainbow trout, *Salmo gairdnerii* Richardson. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, **81,** 285–289.

Rust, M.B. (2002) Nutritional physiology. In: *Fish Nutrition* (Halver, J.E. & Hardy, R.W. eds), Vol. 3, pp. 367–452. Academic Press, Orlando, FL.

Santulli, A., Curatolo, A., Modica, A. & D'Amelio, V. (1988) Time-course changes of plasma lipid levels and lipoprotein pattern after feeding in cultured sea bass, *Dicentrarchus labrax L. J. Fish Biol.*, **32,** 859–867.

Sargent, J.R., Tocher, D.R. & Bell, J.G. (2002) The lipids. In: *Fish Nutrition* (Halver, J.E. & Hardy, R.W. eds), Vol. 3, pp. 181–257. Academic Press, Orlando, FL.

Sheridan, M.A. (1988) Lipid dynamics in fish: aspects of absorption, transportation, deposition and mobilization. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, **90,** 679–690.

Sheridan, M.A., Friedlander, J.K.L. & Allen, W.V. (1985) Chylomicra in the serum of prostprandial steelhead trout (*Salmo gairdnerii*). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.*, **81,** 281–284.

Sire, M.F., Lutton, C. & Vernier, J.M. (1981) New views on intestinal absorption of lipids in teleostan fishes: an ultrastructural and biochemical study in the rainbow trout. *J. Lipid Res.*, **22,** 81–94.

Soivio, A., Nynolm, K. & Westman, K. (1975) A technique for repeated sampling of the blood of individual resting fish. *J. Exp. Biol.*, **63,** 207–217.

Steffensen, J.F. & Lomholt, J.P. (1992) The secondary vascular system. In: *Fish Physiology* (Hoar, H.S. *et al.* eds), Vol. 12A, pp. 185–217. Academic Press, San Diego.

Suarez, R.K. & Mommsen, T.P. (1987) Gluconeogenesis in teleost fishes. *Can. J. Zool.*, **65,** 1869–1882.

Thomassen, M.S., Strom, E., Christiansen, E.N. & Norum, K.R. (1979) Effect of marine oil and rapeseed oil on composition of fatty acids in lipoprotein triacylglycerols from rat blood plasma and liver perfusate. *Lipids*, **14,** 58–65.

Tomas, E., Kelly, M., Xiang, X.Q. *et al.* (2004) *Metabolic and Hormonal Interactions Between Muscle and Adipose Tissue*, pp. 381–385. CABI Publishing, Wallingford .

Weber, N., Klein, E. & Mukherjee, K.D. (2002) The composition of the major molecular species of adipose tissue triacylglycerols of rats reflects those of dietary rapeseed, olive and sunflower oils. *J. Nutr.*, **132,** 726–732.

Yu, Y.-H. & Ginsberg, H.N. (2005) Adipocyte signaling and lipid homeostasis: sequelae of insulin-resistant adipose tissue. *Circ. Res.*, **96,** 1042–1052.

Zheng, X.Z., Torstensen, B.E., Tocher, D.R., Dick, J.R., Henderson, R.J. & Bell, J.G. (2005) Environmental and dietary influences on highly unsaturated fatty acid biosynthesis and expression of fatty acyl desaturase and elongase genes in liver of Atlantic salmon (*Salmo salar*). *Biochim. Biophys. Acta Mol. Cell Biol. L.*, **1734,** 13–24.