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Short-term effects of fish and fish oil consumption on total and high molecular weight adiponectin levels in overweight adults

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Abstract

Objective: Fish or fish oil consumption may increase levels of total and high molecular weight (HMW) adiponectin, a hormone associated with anti-inflammatory and insulin-sensitising effects, however it is not known if the effects of the food and supplement are the same. The aim of this study was to compare the effect of consuming fish and fish oil supplements on plasma total and HMW adiponectin concentrations in overweight human subjects.

Materials/Methods: 29 overweight participants underwent a two week run-in period, followed by a four week isocaloric dietary intervention which provided 1.8g of long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) in the form of either fish or fish oil supplements. Primary outcomes were changes in plasma total and HMW adiponectin. Secondary outcomes were changes in anthropometric variables, plasma insulin and glucose levels, and dietary intakes.

Results: Changes in plasma HMW adiponectin during the intervention period were significantly different between groups (p=0.009). Mean HMW adiponectin increased by 0.29ug/mL in the 'fish' group and decreased by 0.60ug/mL in the 'supplement' group. Similar trends were seen for total adiponectin however these did not reach statistical significance. There were no significant changes in other anthropometric and biochemical variables. Dietary data suggested the 'fish' group significantly increased their fish (p=0.001) and dietary LC n-3 PUFA (p=0.001) consumption over the course of the study.

Conclusions: Short-term consumption of fish and fish oil supplements did not have the same effects on HMW adiponectin levels. The impact of fish intake on HMW adiponectin levels may not be mediated by its LC n-3 PUFA content alone.

Keywords: omega-3, adipocyte hormone, dietary intervention

List of abbreviations used in this manuscript

BMI: body mass index

DH: diet history

DHA: docosahexaenoic acid

EPA: eicosapentaenoic acid

FTO: fat mass and obesity-associated

HMW: high molecular weight

IQR: interquartile range

LC n-3 PUFA: long chain omega-3 polyunsaturated fatty acids

PPARγ: peroxisome proliferator activated receptor γ

SD: standard deviation

SNP: single nucleotide polymorphisms

Introduction

Adiponectin, a hormone secreted by adipocytes, is known to play a role in mediating inflammation, as well as having anti-obesity and insulin sensitising effects [1, 2]. Adiponectin levels are lower in individuals who are obese [3] or type II diabetic [4], and treatment with adiponectin in animal models improves insulin sensitivity and promotes weight loss [2, 5]. Adiponectin circulates in multimers of varying metabolic weights, including high molecular weight (HMW) adiponectin [6]. HMW is thought to be the more physiologically active form of adiponectin, with HMW adiponectin levels found to be a better predictor of insulin sensitivity and other components of the metabolic syndrome than total adiponectin concentrations [7].

There is evidence to suggest that consumption of either fish or fish oil supplements rich in long chain omega-3 polyunsaturated fatty acids (LC n-3 PUFA) can increase adiponectin levels in both animal models [8-11] and humans [12-17]. This effect is thought to be mediated by the activation of Peroxisome Proliferator Activated Receptor γ (PPARγ) by LC n-3 PUFA, which results in increased adiponectin synthesis in adipose tissue [18].

It is currently unclear whether fish and fish oil supplements have the same effect on adiponectin synthesis and secretion. The concept of food synergy proposes that interaction between the bioactive compounds present in foods may be responsible for providing additional health benefits, over and above those provided by the actions of the single nutrients in these foods [19, 20]. In accordance with this concept, research suggests that whole fish may have additional health benefits than those provided by fish oil alone [21, 22].To date, however, only very few studies have compared the effects of fish and fish oil on any health outcome, including circulating adiponectin concentrations. Futhermore, most studies investigating the effects of foods on adiponectin levels have failed to measure changes in HMW adiponectin, despite its known physiological importance.

There is also evidence from recent human reports that single nucleotide polymorphisms (SNPs) in the gene encoding for adiponectin, *ADIPOQ*, can influence circulating levels of adiponectin, with individualscarrying the C allele of the *ADIPOQ* SNP rs266729 have been found to have lower levels of circulating adiponectin than those homozygous for the G allele [23]. Similarly, SNPs in the fat mass and obesity-associated (*FTO)* gene may influence adiponectin levels as well as health outcomes [24, 25], and Caucasian women who were homozygous for the A allele of the *FTO* SNP rs9939609 have been found have lower circulating levels of adiponectin than those homozygous for the T allele [24]. Therefore, such variations have the potential to pre-dispose individuals to altered levels of adiponectin and could thus confound the results of dietary interventions. However,

no known study assessing the effect of fish or LC n-3 PUFA on adiponectin levels has measured SNPs in *ADIPOQ* or *FTO* genes.

The primary aim of this study was to compare the effects of consumption of fish and fish oil supplements, providing a similar amount of LC n-3 PUFA, on total and HMW adiponectin levels in overweight humans. A secondary aim was to determine whether these effects were modified by the participant's ADIPOQ and/or FTO genotype.

Materials and Methods

Study design: A six week pilot study was conducted with overweight adults randomised to one of two parallel arms: fish diet group ('fish') and fish oil group ('supplement'). Participants were block randomised by gender and body mass index (BMI) category (25-30kg/m^2 and 30-35kg/m^2). Both groups underwent a two week run-in period (t= -2-0 weeks), designed to orient them to the study protocol and to observe any reductions in weight following commencement of the study. Following this two week run-in period, both groups commenced the four week dietary intervention (t=0–4 weeks). Primary outcomes were change in total and high molecular weight (HMW) plasma adiponectin concentrations. Secondary outcomes included changes in anthropometric variables, fasting insulin and glucose concentrations and dietary intakes.

Participants were recruited via advertisements sent to University of Wollongong general and academic staff and flyers distributed at University of Wollongong events. *Inclusion criteria:* aged 18–65 years, willing to consume fish, BMI >25 and <37kg/m^2, waist circumference >94cm for men, >80cm for women, generally well.

Exclusion criteria: Diabetes mellitus, impaired renal function, smoking, not weight stable for the past six months, food allergies or habits inhibiting compliance with the study design, illiteracy and inadequate conversational English, currently taking medications including thiazolidinediones, valproic acid, ACE inhibitors, and glucocorticoids, pregnancy/lactation, high consumption of fish (>two serves per week)

Dietary intervention: For six weeks both groups were advised to consume an isocaloric diet for weight maintenance (meeting estimated energy requirements [26] with 1.25 physical activity factor) and consisting of 25% protein, 45% carbohydrate, and 30% fat. Diets referred to low fat staple foods (fruit, vegetables, cereals, lean meat, low fat milk and yoghurt) and small amounts of nuts, seeds, spreads and oils. Due to the effects of changes in alcohol consumption on adiponectin levels [27, 28], participants were advised to maintain their normal level of alcohol consumption over the course of the study. Following the two week run-in period, participants in the 'fish' group were provided with three serves of 135g salmon (Birds Eye Atlantic Salmon Fillets, Simplot Australia), two serves of 66g sardines (adjusted for percentage fish in total canned product; John West Sardines in Tomato Sauce, Simplot Australia) and one serve of 55.1g tuna (adjusted for percentage fish; John West Tuna Tempters Lemon and Cracked Pepper, Simplot Australia) per week for four weeks. Due to an inability to consume sardines, one participant was provided with four serves of 135g salmon and one serve of 55.1g tuna per week. The fish provided was designed to contribute approximately 1.86g of LC n-3 PUFA per day (approximately 812mg Eicosapentaenoic acid [EPA] + 1044mg Docosahexaenoic acid [DHA]). During the dietary intervention period, participants in the 'supplement' group were given three fish oil supplements per day, to provide 1.8g of total LC n-3 PUFA (1055.1mg EPA + 744.9mg DHA; Blackmores Omega

Daily). Participants in the 'supplement' group were not given any specific advice regarding fish consumption.

Diet histories (DH) interviews [29] were collected at t= -2 weeks and t=4 weeks to identify changes from habitual diets. Dietary intake was calculated using the Foodworks nutrient analysis software (Xyris Pty Ltd, Highgate Hill, QLD, Australia, Version 6, 2009), using the 'AUSNUT2007 Brands' and 'AUSNUT2007 Foods' nutrient databases [30]. Fish intake was calculated as grams of fish consumed per week.

All remaining fish and supplements were collected at the end of the study to measure compliance. Returned supplements were used to calculate LC n-3 PUFA intake in the 'supplement' group in addition to dietary LC n-3 PUFA measured by DH interview.

All participants were advised to maintain their normal level of physical activity over the duration of the study. Habitual physical activity was assessed prior to the run-in period (t= -2 weeks) and at the end of the study (t=4 weeks) via the Baecke questionnaire [31].

Anthropometry: Body weight and percentage body fat were measured in an upright position at t= -2, 0, 4 weeks, in minimal clothing and without shoes using scales with a bioelectrical impedance component (Tanita TBF-662). Waist circumference was measured at t= -2, 0, 4 weeks with a flexible tape measure.

Insulin, glucose, plasma fatty acids and adiponectin: were measured in the morning after an overnight fast at t= -2, 0, 4 weeks. Insulin and glucose levels were measured by a quality assured pathology laboratory (Southern IML Pathology), whilst plasma fatty acids were analysed by the Functional Food Group, FOODplus Research Centre, School of Agriculture, Food and Wine, University of Adelaide as described by Tu *et al.* [32]. Plasma total and high molecular weight (HMW) adiponectin concentrations were measured using a multimeric

enzyme-linked immunosorbent assay (Alpco Diagnostics Inc, Salem, NH). All adiponectin concentrations were measured in duplicate and any questionable results were re-tested. Care was taken to ensure all samples from the same participant were measured in the same assay run.

For the SNP analysis in *ADIPOQ* and *FTO* genes, saliva samples were collected for DNA extraction (Oragene, DNA Genotek, USA) at t=4 weeks. Due to the known ethnic variations in the prevalence of the tested SNPs and their functional polymorphisms [33, 34], saliva samples were collected only from participants of Caucasian ethnicity (n=25). Analysis of SNPs in *ADIPOQ* (rs266729) and *FTO* (rs9939609, rs8050136) was performed in duplicate using MALDI-TOF mass spectrometry (Sequenom MassARRAY iPLEX Platform).

Statistical analysis: Data was analysed using SPSS (version 17.0, SPSS Chicago, IL, 2008). Normality of the data was determined using the Shapiro-Wilks test. Mean and standard deviation (SD) of all parametric variables were calculated, whilst median and inter-quartile range (IQR) were calculated for non-parametric variables.

As the run-in period (t= -2–0 weeks) was designed to stabilise measures, reduce the within-participant variation and minimise the possibility of regression to the mean, the anthropometric and biochemical data from this period was not included in the analysis. As the literature suggests that research should focus on the change in adiponectin levels over time rather than single measures at an individual time point [35], changes in total and HMW adiponectin from t=0 to t=4 weeks were calculated and compared between groups via an independent samples t-test and Mann-Whitney test for parametric and non-parametric data respectively. This approach has been previously used in the adiponectin literature [15, 36]. Changes in total and HMW adiponectin from t=0 to t= 4 weeks within groups were calculated

and compared via a paired samples t-test and a Wilcoxan signed ranks test for parametric and non-parametric data respectively

Differences in other biochemical and anthropometric variables over time and between groups were measured via mixed between-within subjects ANOVA for parametric variables. For non-parametric dietary data, differences between groups were measured via a Mann-Whitney test, whilst differences in biochemical and anthropometric variables within groups over the duration of the intervention period (t=0–4 weeks) were measured via Wilcoxon signed ranks test. Differences in dietary variable and the Baecke questionnaire over the duration of the pilot study and between groups were also measured in this way. Due to a violation of the minimum cell frequency assumption of the Pearson's chi-square analysis, Fisher's exact test was used to determine if there was a significant difference between the allelic frequencies of SNPs in *ADIPOQ* and *FTO* in the 'fish' and 'supplement' groups.

Compliance to recommended fish and supplement intake was measured as the number of fish or supplements consumed (calculated from returned products) as a percentage of the number of fish or supplements recommended provided. Mean and SD compliance of the study sample was then determined.

Ethical approval was granted by the University of Wollongong Human Research Ethics Committee and informed consent was obtained for all participants.

Results

Of the n=93 individuals who expressed an interest in the study, n=30 met the inclusion criteria and were able to meet study requirements, and n=28 were present at t=0 and

4 weeks (Figure 1). Total and HMW adiponectin results were excluded for one participant at all time points as a result of implausible findings.

There were no significant differences in total and HMW adiponectin levels between study groups at t = 0 (Table 1). Change in HMW adiponectin over the intervention period was significantly different (p=0.009) between the 'fish' and 'supplement' groups. In the 'supplement' group, there was a significant decrease in HMW adiponectin levels over the duration of the study (p=0.026), whilst there was no change in plasma HMW adiponectin levels in the 'fish'group.. There were no significant changes in total adiponectin over the duration of the study in either of the treatment groups and total adiponectin concentrations were not different between the 'fish' and 'supplement' groups at any timepoint.

The percentage of EPA + DHA in plasma phospholipids increased significantly over the intervention period in both the 'fish' (p=0.001) and 'supplement' groups (p=0.001), but there was no significant difference between the groups at t=4 weeks (p=0.114) (Table 2). There were no significant differences within or between groups in any other anthropometric or biochemical variable. There was also no significant difference between groups in the allelic frequencies of SNPs in *ADIPOQ* and *FTO* genes (Table 3).

The 'fish' group reported significantly increasing fish intake (p=0.001), and were consuming significantly higher amounts of fish than the 'supplement' group at the end of the intervention (p<0.001). Similarly, significantly higher intakes of LC n-3 PUFA from the diet were reported by the 'fish' group (p<0.0001), however there was no significant difference between the total LC n-3 PUFA intake from both dietary and supplement sources combined between the 'fish' and 'supplement' groups (p=0.285). During the intervention both the 'fish' and 'supplement' groups reported a reduced percentage energy intake from total and saturated fat (time effect: p=0.005, p=0.001 respectively) (Table 4). A significant interaction effect was also seen for percent energy from polyunsaturated fat (p=0.001).

Mean and SD compliance for the 'fish' and 'supplement' groups was 87.51±16.54% and 90.23±11.20%, respectively.

Discussion

The results of this study suggest that short-term consumption of fish and fish oil supplements do not have the same effect on HMW adiponectin levels in overweight humans. Over the course of a dietary intervention which incorporated the same amount of LC n-3 PUFA provided by either fish or fish oil, a significantly different change in HMW adiponectin was found between groups; This was due to a small increase in HMW adiponectin in the 'fish' group, whilst the 'supplement' group exhibited a significant decrease in HMW adiponectin concentraitons following the dietary intervention, there was no change in HMW adiponectin in those receiving the same level of n-3 LCPUFA from fish for the same period. A similar pattern was seen for total adiponectin; however this did not reach statistical significance. These changes occurred whilst factors known to be associated with adiponectin levels, such as weight and insulin levels [37], remained relatively constant.

This data suggests that the health benefits of fish may not be limited to the LC n-3 PUFA content alone. It is currently not known why differential effects on HMW adiponectin were seen for fish and LC n-3 PUFA supplement consumption, and whilst this study was not designed to test mechanisms for change, some possibilities can be proposed. As a whole food, fish consists of a variety of additional nutrients and bioactive ingredients which could impact upon health. Consumption of fish protein has been linked to improvements in insulin sensitivity [38] and insulin response [39]. Furthermore, other components present in fish such as selenium and vitamin D have also been associated with a range of health benefits in humans [40, 41]. Whilst there has been no research conducted specifically investigating the

influence of consumption of these nutrients on adiponectin levels, due to their known health benefits and as the intervention diets in the present study were matched for total LC n-3 PUFA content, it is reasonable to suggest that compounds such as these, in addition to the LC n-3 PUFA, may have played a role in the changes in HMW adiponectin levels. The differential effects of fish and fish oil found in the present study are supported by research by Cobiac *et al*. [21], who found improvements in haemostatic factors in hyperlipidemic men following fish, but not fish oil, consumption.

No previous research has compared the effects of fish and fish oil supplements on HMW adiponectin and only one study has done so using total adiponectin as an outcome. Ramel *et al.* [42] provided participants with salmon, cod, fish oil supplements or a control diet of chicken over 12 weeks and found a reduction in total adiponectin in all groups, with no significant difference between groups. However, this study did not investigate changes in HMW adiponectin levels, which have been found to increase in concentration post-weight loss even when no changes in total adiponectin were seen [43]. Furthermore, unlike the present study, Ramel *et al.* [42] did not match the LC n-3 PUFA provided by the salmon and fish oil diets, making comparisons between the whole food and supplement problematic.

Whilst there is a paucity of literature comparing the effects of fish and fish oil on HMW adiponectin, several studies have examined the impact of either fish or fish oil consumption on total adiponectin with varying results. Guebre-Egziabher *et al.* [12]; Kondo *et al*. [16]; and Lara *et al.* [14] found increases in total adiponectin levels following fish consumption, whilst Krebs *et al.* [13]; Sneddon *et al*. [15]; and Gammelmark *et al*. [17] found similar results after supplementation with fish oil. A limitation of these studies however, is that none examined the effect of fish consumption on HMW adiponectin. Only one previous study has examined the influence of either fish or fish oil supplements on HMW adiponectin, and did not see a significant effect of fish oil [44]. Given the known biological importance of

HMW adiponectin, it is important to investigate this component in addition to total adiponectin, as non-significant changes in total adiponectin could mask a significant change in the HMW multimer, as was found in the present study.

In the present study, a significant decrease in total and HMW adiponectin concentrations was found in the 'supplement' group. This contrasts with the results of previous studies which have found increases in total adiponectin levels following supplementation with fish oil [13, 15, 17]; however this may be the result of methodological issues in previous research, such as the use of an ad libitum study diet [15], and failing to measure dietary LC n-3 PUFA intake [13, 15, 17]. A strength of the current study was that dietary variables were controlled for through a prescribed study diet, which were confirmed through dietary assessment. The DH data indicated the 'fish' group consumed significantly higher amounts of LC n-3 PUFA from the diet than the 'supplement' group at 6 weeks (Table 2). The inclusion of total LC n-3 PUFA consumed (calculated from returned supplements) suggested that both groups consumed similar amounts of LC n-3 PUFA from dietary and supplement sources as planned. This was supported by the plasma fatty acid analysis, which found no difference in levels of omega-3 or EPA + DHA between groups at the end of the intervention.

Genetic analysis performed in the present study confirmed that there was no difference between the allelic frequency of SNPs in an adiponectin encoding gene and a gene associated with risk of obesity in the 'fish' and 'supplement' groups. These SNPs, which have been associated with alterations in circulating levels of adiponectin and risk factors for the metabolic syndrome and its associated diseases [23, 24, 34] could potentially confound the results of this study if variations existed between tested groups. However, similar genotypic and allelic frequencies of the tested SNPs found in both study groups suggests the findings of the present study were due to dietary changes rather than genetic variation

between groups. This is a strength of the current study, as no previous studies have investigated genetic variation between groups.

This study was limited by its small sample size and the short time period of the dietary intervention. However, as no previous research has compared the effects of fish and fish oil on HMW adiponectin, or indeed, many other health outcomes, this study has helped to establish proof of concept in this area. The lack of a separate control group is also a limitation of this study; however the run-in period addressed some of this problem by eliminating the effect of weight loss following commencement of dietary advice.

This study, which was the first to compare the effects of fish and fish oil supplements on total and HMW adiponectin, has shown that short-term consumption of fish and supplements do not have the same effect on this hormone. This finding was made in the absence of confounding factors such as dietary and genetic variations between groups. Whilst the changes in HMW adiponectin found in this study were relatively small, the differing patterns seen with fish and fish oil consumption imply dissimilar biological effects which necessitate further investigation.

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The authors declare they have no conflict of interest

Author contribution:

EN designed, organised and led the study, data collection and analysis and preparation of the manuscript. BM, YP, MB and LT contributed to critical discussion of the study design and analysis and critical revisions of the manuscript. FF provided critical discussion of genetic procedures and carried out the genetic analysis. All authors approved the final version of the manuscript.

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Table 1: Mean ± SD [median (IQR)] change in total and HMW adiponectin levels from t=0 to t=4 weeks

	'Fish' (n=12)				'Supplement' (n=14)				p-value (between groups)	
	t=0	t=4	Change (t=0 to t=4 weeks)	p-value (within group change)	t=0	t=4	Change (t=0 to t=4 weeks)	p-value (within group change)	t=0	Change
Total adiponectin (ug/mL)	6.93 ± 2.82 [6.69 (4.43 – 8.38)]	7.74 ± 3.36 [7.57 (4.97 – 10.10)]	0.81 ± 1.95 [0.34 (-0.37 – 1.01)]	0.180[1]	6.60 ± 2.34 [6.25 (4.31 – 8.47)]	6.46 ± 2.51 [5.96 (4.34 – 8.91)]	-0.14 ± 2.05 [0.51 (-1.19 – 1.44)]	0.778[2]	0.746[3]	0.860[4]
HMW adiponectin (ug/mL)	3.63 ± 2.45 [3.62 (1.25 – 5.00)]	3.92 ± 2.96 [3.62 (1.07 – 5.32)]	0.29 ± 0.97 [0.01 (-0.25 – 0.40)]	0.321[1]	3.39 ± 2.07 [2.63 (1.61 – 4.96)]	2.81 ± 1.80 [2.12 (1.30 – 4.25)]	-0.58 ± 1.18 [- 0.63 (-0.99 – -0.22)]	**0.026[2]**	0.789[3]	**0.009[4]**

[1]Paired samples t-test [2]Wilcoxon signed ranks test

[3]Independent samples t-test [4]Mann-Whitney test

Table 2: Anthropometric and biochemical variables at t= -2, 0 and 4 weeks

	'Fish'			'Supplement'			p-value fish vs supps (between groups)
Variable	**Mean (med.)**	**SD (IQR)**	**p-value fish (within group)**	**Mean (med.)**	**SD (IQR)**	**p-value supps (within group)**	
Males|Females (*n*)	5|10			5|9			
t= -2 wks	4|10			5|9			
t=0 wks	4|10			5|9			
t=4 wks							
Weight (kg)							
t=-2 wks	83.11 (80.25)	13.74 (71.15-89.88)		83.72 (80.50)	13.96 (71.60–100.55)		
t=0 wks	82.82 (79.60)	13.72 (70.90-90.35)		82.80 (79.05)	13.55 (70.78–98.68)		1.000[3]
t=4 wks	82.78 (78.45)	13.91 (71.95-90.85)	0.861[5]	82.55 (78.45)	13.86 (70.43-98.33)	0.509[5]	0.966[4]
BMI (kg/m^2)							Time:0.480
t=-2 wks	28.93	2.98		29.26	3.00		Group:0.947
t=0 wks	28.83	2.98		28.95	2.93		Interaction:
t=4 wks	28.81	3.01		28.84	2.93		0.647[6]
Waist (cm)							Time:0.083
t=-2 wks	95.76	9.57		98.57	10.70		Group:0.538
t=0 wks	94.96	10.00		97.61	10.80		Interaction:
t=4 wks	94.86	9.90		97.11	10.84		0.253[6]
Body fat (%)							Time:0.265
t=-2 wks	37.28	7.16		35.97	7.27		Group:0.536
t=0 wks[2]	36.89	7.13		35.09	7.86		Interaction:
t=4 wks	36.57	6.5		34.74	8.10		0.955[6]
Glucose (mmol/L)							
t=-2 wks	5.34 (5.30)	0.38 (5.10-5.60)		5.21 (5.25)	0.38(4.95-5.23)		
t=0 wks[1]	5.32 (5.30)	0.52 (5.05-5.65)		5.28 (5.25)	0.48(4.88-5.80)		0.819[4]
t=4 wks	5.26 (5.10)	0.38 (4.95–5.70)	0.408[5]	5.14 (5.20)	0.43(4.78-5.55)	0.178[5]	0.427[3]
Insulin (mU/L)							
t=-2 wks	9.28 (8.10)	4.75 (6.05-13.05)		11.86 (9.80)	7.24(6.90-15.98)		
t=0 wks[1]	9.28 (7.10)	5.12 (5.80-12.00)		12.23 (10.65)	9.00(7.18-12.70)		0.302[3]
t=4 wks	9.20 (8.10)	3.38 (6.85-11.45)	0.937[5]	10.63 (9.15)	9.15(5.68–14.03)	0.198[5]	1.000[3]
EPA + DHA(%)							
t=-2 wks	4.14 (4.03)	0.64 (3.71-4.59)		4.97 (4.75)	0.88 (4.39-5.76)		
t=0 wks[1]	4.94 (4.81)	1.37 (4.04–5.41)		5.33 (5.07)	1.54 (4.27-5.89)		0.550[3]
t=4 wks	8.12 (8.90)	2.08 (5.90–9.74)	0.001[5]	9.30 (9.54)	1.53(8.37-10.31)	0.001[5]	0.114[3]

[1]Data available for n = 27 participants (n=13 fish, n=14 supplements)

[2]Data excluded for n = 1 participant due to machinery malfunction (data available for: n=13 'fish', n=14 'supplements')

[3]Mann-Whitney test [4]Independent t-test

[5]Wilcoxon signed ranks test [6]Mixed between-within subjects ANOVA

Table 3: Allelic frequencies of SNPs in *ADIPOQ* (rs266729), and *FTO* (rs9939609, rs8050136) between 'fish' and 'supplement' groups

	'Fish'		**'Supplement'**		**P-value[1]**
SNPs	**Genotypes**		**Genotypes**		
rs266729	CC	GC/GG[2]	CC	GC/GG[2]	
(*ADIPOQ*)	7	6	5	7	0.695
rs9939609	TT	AT/AA[3]	TT	AT/AA[3]	
(FTO)	3	10	4	8	0.673
rs8050136	CC	CA/AA[3]	CC	CA/AA[3]	
(FTO)	3	10	4	8	0.673

[1]Fisher's Exact Test

[2]G allele associated with decreased levels of adiponectin [43]

[3]A allele associated with increased risk of obesity [27]

Table 4: Reported daily dietary intake and physical activity levels at t= -2 and 4 weeks

	'Fish'			'Supplement'			p value fish vs supps (between groups)
Variables	**Mean (med.)**	**SD (IQR)**	**p-value fish (within group)**	**Mean (med.)**	**SD (IQR)**	**p-value supps (within group)**	
Fish (g)							
t=-2 wks	24.75 (20.41)	18.80 (10.15-38.42)		38.53 (36.09)	22.39 13.99-31.01)		0.064§
t= 4 wks	90.98 (87.24)	21.90 (84.58 – 99.18)	**0.001[3]**	30.31 (20.84)	29.54 (13.99 – 31.01)	0.140[3]	**0.000[1]**
LC n-3 PUFA (mg)							
t=-2 wks	359.06 (211.78)	341.07 (150.20 – 499.26)		502.81 (493.44)	295.04 (230.02 – 662.50)		0.051[1]
t=4 wks (diet only)	1901.16 (1925.14)	328.11 (1898.20 – 1957.03)		339.65 (264.83)	213.67 (203.78-450.61)		**0.000[1]**
t=4 wks (diet + supp)	1901.16 (1925.14)	328.11 (1898.20 – 1957.03)	**0.001[3]**	1990.73 (1982.53)	206.32 (1800.63– 2194.98)	**0.030[3]**	0.285[2]
Energy (kJ)							
t=-2 wks	8173.06 (8029.36)	1740.14 (6581.85-9435.74)		8584.13 (9148.50)	2190.11 (6905.50-10136.46)		0.962[5]
t=4 wks	7536.06 (7017.61)	2287.46 (6414.04 – 7888.74)	0.074[3]	7680.67 (6960.58)	2514.00 (5840.40 – 8892.84)	0.056[3]	0.769[1]
Protein (%E)							Time: 0.553
t=-2 wks	19.58	4.10		21.03	3.53		Group: 0.321
t=4 wks	20.20	2.53		21.33	5.06		Interaction: 0.838[4]
Total fat (%E)							T Time: **0.005**
t=-2 wks	31.50	4.18		30.61	6.75		Group: 0.186
t=4 wks	29.35	5.35		25.14	7.14		Interaction: 0.197[4]
SFA (%E)							Time: **0.001**
t=-2 wks	12.02	3.17		10.57	3.62		Group: 0.526
t=4 wks	8.99	2.09		9.20	2.90		Interaction: 0.177[4]
PUFA (%E)							Time: 0.505
t=-2 wks	5.29	1.48		5.42	1.94		**Group: 0.021**
t= 4 wks	6.84	1.27		4.37	1.56		**Interaction: 0.001**[4]
MUFA (%E)							Time: 0.060
t=-2 wks	11.88	2.56		12.26	4.08		Group: 0.331
t= 4 wks	11.73	3.17		9.39	3.30		Interaction: 0.088[4]
CHO (%E)							**Time: 0.036**
t=-2 wks	43.63	7.15		44.04	5.62		Group: 0.389
t= 4 wks	44.81	5.18		47.96	6.53		Interaction: 0.244[4]
EtOH (%E)							
t=-2 wks	3.40 (2.53)	2.64 (1.73 – 5.33)		2.11 (1.36)	2.24 (0.004 - 3.95)		0.186[1]
t= 4 wks	3.20 (2.48)	2.79 (1.70 – 3.65)	0.875[3]	2.87 (2.26)	3.19 (0.19 – 4.52)	0.279[3]	0.603[1]
Baecke questionnaire							Time: 0.578
t=-2 wks	7.80	1.44		7.09	1.18		Group: 0.302
t= 4 wks[6]	7.65	1.33		7.37	1.11		Interaction: 0.072[4]

[1]Mann-Whitney test
[2]Mann-Whitney test (compared to dietary LC n-3 PUFA at t= -2 weeks)
[3]Wilcoxon signed ranks test
[4]Mixed between-within subjects ANOVA
[5]Independent t-test
[6]Data available for n=27 participants (n=13 'fish', n=14 'supplements')

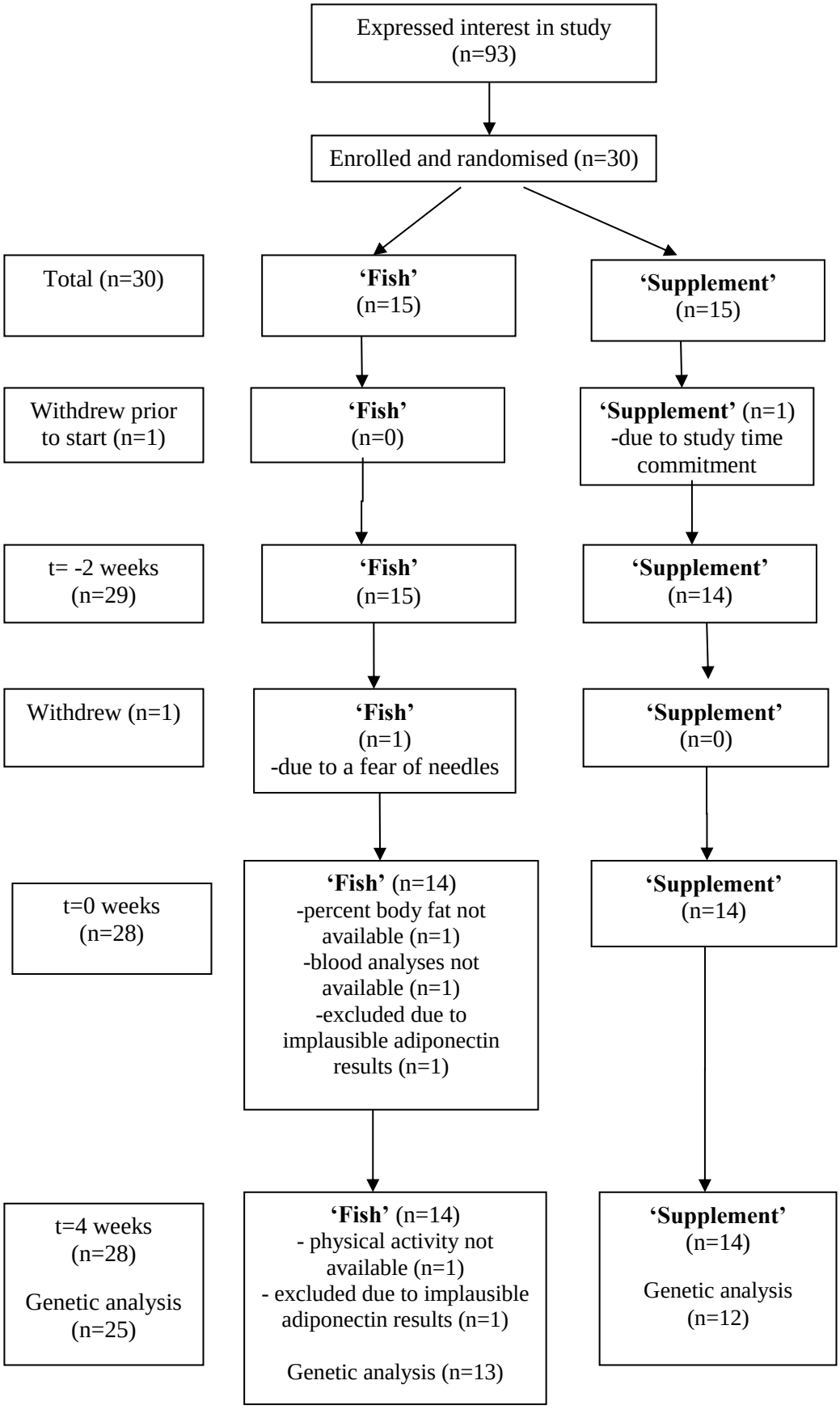

Figure 1: Enrolment, randomisation and available data for study participants over the duration of the study