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The Effect of Long Chain N-3 PUFA on Obese Individuals - Desk Review

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Abstract

Context: Current recommendations for counteracting the action of obesity advocate the consumption of a healthy diet and participation in regular physical activity. Studies have indicated towards a potential role of long-chain omega-3 polyunsaturated fatty acids (LC N-3 PUFA) in a number of effects which ameliorate the condition of obesity.

Objective: To assess the effect of LC N-3 PUFA (Docosahexaenoic acid and Ecosapentaenoic acid) on obese individuals.

Data sources: Using Cochrane guidelines, electronic databases- Google Scholar and PubMed-NCBI were searched for evidence from the last 10 years (March 2007-17).

Study selection: Randomized Controlled Trials (RCT) based on LC N-3 PUFA supplementation involving obese adult were included.

Result: The association between change in body weight/ body mass index (BMI)/ waist circumference and fish oil supplementation in 31 RCTs involving overweight or obese adults was investigated. Of the 31 studies, 9 did not provide any evidence for the biomarkers of LC N-3 PUFA intake like EPA/DHA fatty acids plasma concentration and N-3 FA percentage in serum phospholipids. The preliminary evidence suggested that LC N-3 PUFA supplementation combined with energy-restricted diets or exercises prevents weight regain. Treatment groups showed a higher drop in BMI and body fat percentage (0.24kg/m2; 0.49%) than controls.

Conclusion: While there is growing evidence that LC N-3 PUFA can improve body composition, contradictory findings have also been reported. There is an urgent need for long-term studies and meta-analysis in this area of research to generate conclusive evidence on the effects of LC n-3 PUFA supplementation on obesity.

Keyword: Obesity; LC N-3 PUFA; Fish oil; Weight loss; Body weight; Body mass index; Anti-inflammatory biomarkers; CVD risk

Abbreviations: EPA: Eicosapentaenoic Acid; DHA: Docosahexaenoic Acid, LC N:3 PUFA: Long Chain Omega 3 Polyunsaturated Fatty Acids: BW : Body Weight; BCA: Body Composition Analysis; HR: Heart Rate; WC: Waist Circumference; CVD: Cardio Vascular Disease; HRV: Heart Rate Variability; HOMA-IR: Homeostatic Model Assessment-Insulin Resistance; SBP: Systolic Blood Pressure; DPB: Diastolic Blood Pressure; PCOS: Polycystic Ovarian Syndrome; RCT: Randomised Control Trial; T2DM: Type 2 Diabetes; HC: Hypo Caloric Diet; BMI: Body Mass Index; CRP: C: Reactive Protein; IL:6: Interleukin 6; WHR: Waist to Hip Ratio; HDL: High Density Lipoproteins; LDL: Low Density Lipoproteins; VLDL: Very Low Density Lipoproteins; TC: Total Cholesterol; TAG: Triacyl Glyceride

Introduction

Globally, 2.1 billion people are overweight [1]. Worldwide obesity prevalence rate has more than doubled since 1980 [1]. The 2013 Global Burden of Disease study, published in May 2014, showed that 37% of men and 38 % of women had Body Mass Index ≥ 25 kg/m2. It stands for a rise of 28% adult and 47% children since 1980. Considering the demographic factors, it has been observed that the prevalence of overweight individuals is higher in urban settings as compared to rural settings [2].

A cross: sectional study was conducted by Indian Council of Medical Research (ICMR): ICMR: INDIAB (Indian Diabetic Study), which suggested that India, the second most popular country in the world, is currently experiencing a rapid epidemiological transition from underweight to obesity [3]. The National Family Health Survey:4 (NFHS:4) also reported that in India, obesity (BMI ≥ 25kg/m2) was more prevalent in the urban areas (Madhya Pradesh, West Bengal, Chhattisgarh, Orissa) and in higher socio: economic groups as compared to the rural areas, especially among women (Men: urban: 26.6 vs. rural: 14.3%; Women: urban: 31.3 vs. rural: 15.0%) [4-6]. Worldwide, Indians were found to have the highest predisposition to abdominal obesity and accumulation of visceral fat. In context of north India (New Delhi), the overall

prevalence was categorised in terms of generalized obesity (50.1%), to that of abdominal obesity (68.9 %) [5,7].

With the increase in urbanization, poor dietary behaviours [8-9] compounded by decreased levels of physical activity [10-11] has created an obesity: prone environment. This adds on to the prevalence rate of obesity, especially in the developed countries. Genetic susceptibility combined with chronic positive energy balance due to unhealthy lifestyle behaviours and environmental factors are considered as the key features in the development of adiposity [8,11].

Current recommendations from most public health bodies for reducing body weight are based on intensifying physical activity and eating a healthy balanced diet. However, many people are facing difficulty complying with these lifestyle changes, particularly over the longer duration. Hence, despite widespread recommendations to improve diet and physical activity habits the prevalence of obesity continues to rise. Thus, there is an interest in exploring dietary options which could help in keeping body weight in check. One such option may be use of Long Chain Omega:3 fatty acids (DHA & EPA) in or along with our diets [13]. As per WHO, globally, adequate daily intake suggested for omega 3 fatty acid is 1:2 % of total energy i.e. 2:4gm/day for adults [14-15].

Pharmacokinetics of omega 3: A study conducted by Rusca et al. [16] in the year 2009 showed a 2.0fold and 9.3fold rise in plasma DHA and EPA concentrations on consumption of 3 fish oil (FO) capsules (1g each) daily for 28 days. The half: life was found to be 4.8 days and 10.3 days for EPA and DHA respectively by Martijn et al. [17] study. This study was done on 58 overweight men, which were randomised in 4 groups getting an intervention of placebo (9g/d) vs. FO (3g/d) + Placebo (6g/d) vs. FO (6g/d) + Placebo (3g/d) vs. only fish oil (9g/d) for 1 year with 6-month washout period.

Metabolic changes in obesity

Obesity or adiposity is all linked with the alteration in the adipocytokine levels. This is regulated by the action of adiponectin hormone which helps in the metabolism of fat [17-18]. Moreover, dysregulated production of adipocytokine plays a role in the pathogenesis of obesity: associated metabolic syndrome like Cardiovascular Disease (CVD), type 2 diabetes, cancer, inflammation etc [1,8,19]. It has been concluded in several studies that lower levels of adiponectin up: regulate the activity of pro: inflammatory cytokines and increase insulin resistance, oxidative stress, leptin levels and serum C: reactive protein [17,19]. Peroxisome proliferators: activated receptor: γ (PPAR: γ) is a nuclear transcription factor which is highly expressed in the activity of white adipose tissue [19]. It binds to response elements in target gene promoters and regulates the expression of numerous adipose: specific genes [17,19]. A direct relationship between PPAR: γ activity and adipokine differentiation, and its inverse relation to BMI has been found in several observational studies [8,19-20]. Overall, for managing obesity and its related consequences; it is of prime importance to correct the altered activity of adiponectin and other hormones related to adiposity in the body.

In context of Long Chain Omega:3 Polyunsaturated Fatty Acids (LC N:3 PUFA) and its association with obesity, higher levels of LC N:3 PUFA in a normal weight individual than an obese individual have been reported by Jump et al. [21]. This Weight loss mechanism includes, down: regulating the transcription of lipogenic genes and up: regulating the genes that promote lipid oxidation, thermo: genesis, plasma adiponectin, leptin and gherlin levels and anti: inflammatory biomarkers (TNF: a, CRP, IL:6, PPAR: γ activity, etc.) [21-23]. As a result of this, there is a shift in metabolic profile toward one that favours increased fat oxidation and reduced fat deposition which ultimately leads to weight management.

Method

This desk review was conducted by 2 research assistants. To identify relevant studies two search engines Pub Med and Google scholar were used from January through March 2017. Table 1 lists the inclusion and exclusion criteria used in the study. The systematic search identified 487 titles and abstracts of which the full texts of 104 potentially eligible articles were critically appraised. Out of all, 31 studies met the inclusion criteria and were included in the review.

Table 1: Study Selection Criteria.

PICOS	Inclusion Criteria	Exclusion Criteria
Participants	Human subjects (≥ 18yr of age) and body mass index (BMI) ≥ 25kg/m2)	Children adolescents' pregnant women people with any chronic disease
Intervention	DHA/ EPA/ DHA & EPA ≥ 4 weeks;	Omega 6 or Short chain omega 3
Comparison	Placebo or any other Weight loss diet	
Outcome	Reduction in BMI / Body Weight/ Waist Circumference	Any Other
Study setting	Intervention studies	Observational studies

Search strategy

As per the Cochrane collaboration, a search strategy was followed from title-based screening to abstract screening and finally screening full text of the articles. The screening was based on the inclusion and exclusion criteria, which were formed at the beginning of the study. For this review, the relevant records were obtained by using the terms "fish: oil", "omega 3", "DHA or docosahexaenoic acid", "PUFA or polyunsaturated fatty acid" and "N:3 polyunsaturated fatty acids" (Figure 1).

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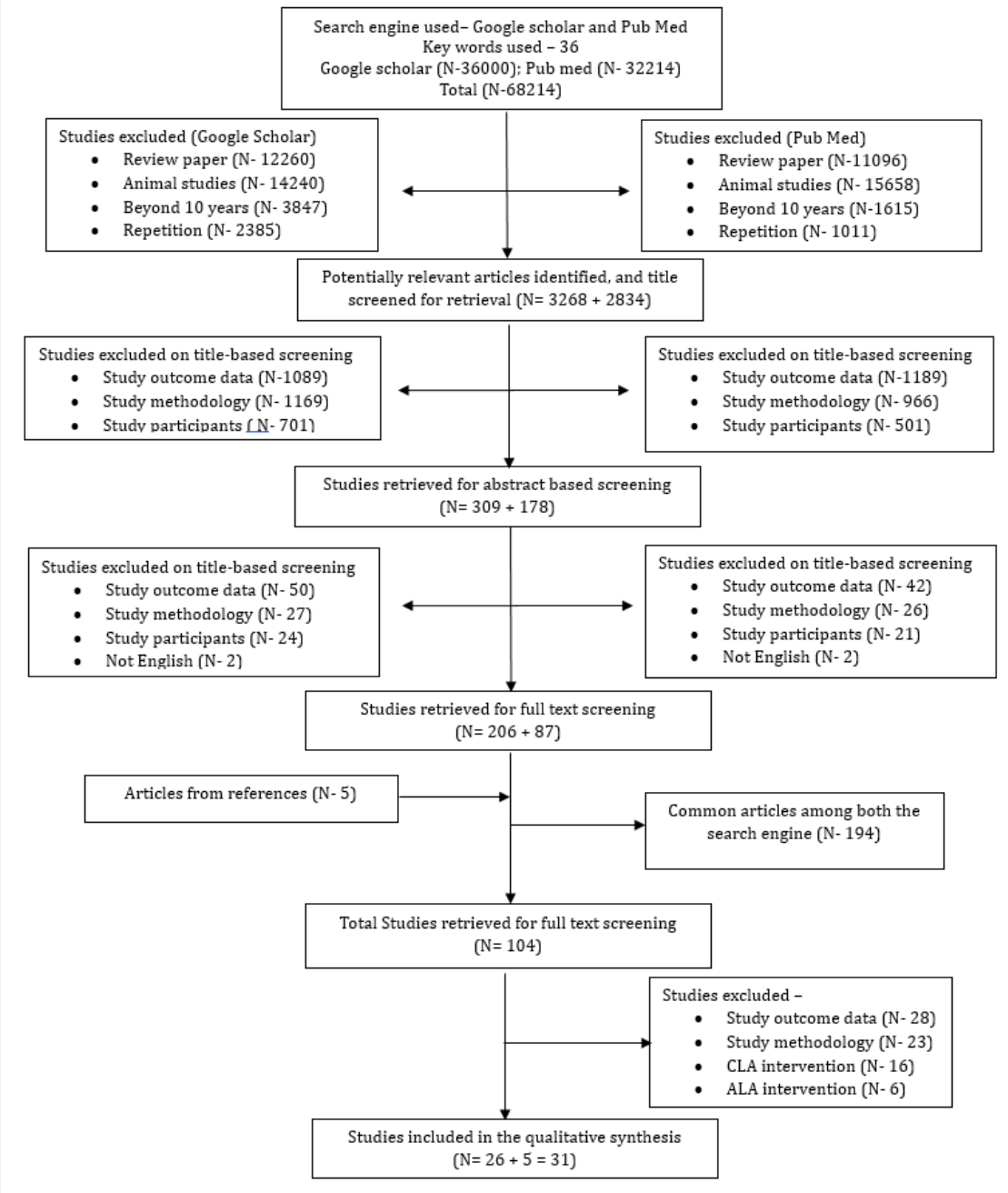

Figure 1: Consort flowchart.

Result

After exclusion according to our criterion, 104 studies were retrieved for full: text screening. Of these, 31 studies met the inclusion criteria among which majority of them were 28 parallel studies with double blind placebo trial and rest 3 studies were crossover studies. In more than half of the studies, the results were interpreted in the form of body weight (BW), waist circumference (WC) and BMI. The association of weight loss in the context of change in Leptin, Gherlin and Adiponectin levels is also illustrated in 9 studies. Beside this, change in inflammatory biomarkers; insulin resistance and lipid profile were also measured that are linked to obesity.

Change in body weight and other parameters of body composition

There were 13 studies whose prime focus was towards the weight lowering action of LC N:3 PUFA supplementation, out of which 5 studies reported a significant effect of DHA: EPA in the treatment of obesity as a reduction in body weight. The first Randomized control trial in healthy overweight patients was published in 2007 by Parra et al. [21,24], this was a result from

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same SEAFOO plus young study. The effect of LC N:3 PUFA on body weight was assessed by making a comparison between 4 experimental groups: no seafood vs. cod fish vs. salmon fish vs. fish oil capsule which were randomised among 324 participants. The trial was conducted for 8 weeks along with the addition of lean meat, 30% energy restriction in diet and increase in physical activity level. The result demonstrated that caloric restriction in the diets induced an average weight loss of 5.8 ± 3.2%, which was statistically significant in all treatment groups and slightly higher in the dietary groups including seafood or fish: oil capsules [21,24-25].

Another RCT (study by Munro et al. [26-27]) on overweight individuals investigated the effect of fish oil (2.1g/day) in comparison to that of Sunola oil (placebo) along with energy restriction diet. At 4 weeks (the intervention phase), the mean weight loss was :6.54kg (26.9%) for Placebo group and :6.87kg (27.7%) for fish oil group. Whereas at week 14 (after 10 weeks of maintenance phase in which participants were given normal diet), there was a further mean decrease in weight, :1.57kg (1.85%) for PB and :1.69kg (:1.9%) for FO. On the top of it, a reduction in fat mass by 8.95% (-3.43kg) in placebo group to that of 9.76% (:3.8kg) in fish oil group followed by increase in fat free mass of 0.67% (0.29kg) for placebo to that of 0.51% (-0.36 kg) in fish oil was also found.

Hardena et al. [28] through their study explained that body mass composition was not affected by treatment, although a fall in body weight in the DHA group approached statistical significance (P = .029). Considering the individual effect of DHA in fat reduction, Neff et al. [29] (n=49) showed a significant reduction of abdominal (p=0.007) and gluteal fat (p=0.001) on supplementation of algal DHA (2g/day) for 4 months as compared to soyabean oil as placebo. Cussons et al. [30] and Crochemore et al. [31] investigated the effect produced by administration of 4g/day of fish oil and 1.5: 2.5g/day of fish oil respectively. The former study was conducted on polycystic ovarian syndrome women whereas the latter was on postmenopausal Type 2 Diabetic patients. The significant decrease in levels of waist circumference, body weight, BMI and fat mass were observed in the fish oil group of PCOS women. The greater percentage of weight loss was found in B group (1.5g/d) as compared to the other groups in later study.

Unlike the studies mentioned above, 4 studies showed contradictory findings. Wong et al. [32] conducted a study on 27 healthy overweight individuals for 16 weeks (12 weeks intervention phase + 4 weeks of maintenance phase). The randomization was done between 2 groups (control and fish oil (4g/ day) group), and no considerable difference in weight loss between the 2 group was found. On the other hand, Gammelmarka et al. [33] in their study also showed the same outcome where they supplemented their one group by fish oil (1.1g/day) and other by olive oil. In the same manner Defina et al. [34] and Tapsell et al. [35] in their studies found no significant difference in reduction of body weight among the groups.

Effect on adeponectin, leptin and gherlin levels

It was illustrated in the study by Wong et al. [36] (n=27) that there is a significant increase in the adiponectin levels in group supplemented with omega 3 source as compared to control group. In context of dosage to time effect, Mohammadi et al. study [37] in which supplementation of fish oil was given to PCOS women (n=64), at the rate of 4g/d for 8 weeks, there was an increase in adiponectin level by 19.5% in FO group. On the other hand, an increment of 4.5% (+0.37µg/ L) was observed in the crossover: canola oil multicentre trial (COMIT) by gravel et al. [38], in canola +¬ DHA group compared to other 4 interventional groups. A similar trend was marked in the study by Gammelmark et al. [33], where the level raised by 0.55µg/ ml in the FO group after 6 weeks.

Gherlin and leptin were found to be having a direct and inverse relationship with the erythrocyte level of DHA + EPA respectively [19]. A comparison was made between the3 experimental groups (Hypo caloric diet+ placebo (control) vs. HC + fish vs. fish + Omega:3 capsules) by Tapsell et al. [35] in their study [39], where group B and C showed a significant increase in the erythrocyte DHA levels followed by reduction in leptin levels (p>0.001).

For comparing the effect of type of fish on leptin and gherlin levels, SEAFOO plus YOUNG study [21] illustrated that there is a potential increase in gherlin and decrease in leptin levels in salmon group followed by supplemented group. Leptin in long term-controlled trial, is significantly related with fullness and reduction in desire to eat [40].

Effect on CVD risk factors: blood pressure, heart rate, serum phospholipids content

There are total of 13 studies which focus on the potential effect of DHA: EPA in reducing the risk factors associated with cardiovascular disease. Out of them, 8 are based on change in total cholesterol, Low density lipoprotein, Triglycerides, High density lipoprotein levels; 2 for change in blood pressure levels; and 3 includes the result based on heart rate variability. Ramel et al. [21], showed a decrease in Systolic blood pressure (:4.4 ± 8.6 mmHg, P < 0.001) and Diastolic Blood Pressure (:4.1 ± 7.4mmHg, P < 0.001) with salmon (P=0.032) and fish oil (P = 0.044) groups more significant than other group, as they both are rich in omega 3 content. The similar results were demonstrated by Cusson et al. [30].

Cardiovascular risk is directly proportional to the levels of serum phospholipids (TG, LDL, and VLDL). Based on the study by Gunnarsdottir et al. [41] (SEAFOO plus YOUNG study) and Munro et al. [26,27], a significant reduction in the total cholesterol (TC), total triglycerides (TG), low density lipoproteins (LDL) was stated in group fed with higher

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concentration of omega 3 (DHA + EPA). Also, it has been proven through the theory of gene regulation that on supplementation of fish oil there is rise in level of HDL in both the studies [26,41]. A significant reduction of serum levels of total cholesterol (A: 42.8%, B: 54.1%, C: 46.1%), LDL: cholesterol (A: 50%, B: 50%, C: 38.5%), and TG (A: 57.1%, B: 64.3%, and C: 53.8%) among the groups was showed by Crochemore et al. [31]. An increase of serum HDL concentration by 42.9%, 50%, and 38.9% in Group A, B, and C, respectively was observed.

A positive association between N:3 PUFA intake and Heart Rate Variability has been reported in adults for managing the high risk for CVD [42]. There was a significant oil treatment X time interaction (P>0·008), as a result of this there was a reduction in resting and exercise HR in the subjects. In addition to this FO intervention had improved HRV by increasing high: frequency power, representing parasympathetic activity, compared with placebo (P=0.01).

Rise in levels of anti: inflammatory biomarkers

A significant treatment effect was observed for plasma CRP (C: reactive protein) concentrations (one of the anti: inflammatory biomarker) (P = 0.04), in canola DHA oil supplemented group only [35]. Ramel et al. [43] and Parra et al. [24] in SEAFOO plus YOUNG study showed the effect of omega 3 in reduction of hs:CRP & Interleukin:6 concentration (high: sensitivity CRP= :32.0%; IL:6= :18.4%) and oxidative stress respectively. The rise in levels of anti: inflammatory cytokines was also observed in the study by Kabir et al. [39] & root et al. [44], where they supplemented Fish oil (FO) approx. at 1.g/d for 8 and 4 weeks respectively. On the contrary, no overall effect was seen in the study by Gammelmarka et al. [33] where same amount of FO was intervened for 6 weeks.

In treatment of type 2 diabetes mellitus: lowering glucose and HOMA:IR levels

Ramel et al. [45] published in his study, that fasting insulin and Homeostatic model assessment - insulin resistance (HOMA:IR) were significantly lower in the fish oil group than in the control group at endpoint (16.4% and 17.2% respectively) through his randomized double-blind control trial. Tapsell et al. [35] & Cusson et al. [30] proposed the same result. Omega:3 fatty acids significantly decreased glucose (by 11.4%, p< 0.001), insulin levels (by 8.4%, p<0.05), and HOMA:IR (by 21.8%, p<0.001) compared with placebo in Rafraf et al. [46] study (Table 2).\

Table 2: Overview and Characteristics of the Included Studies.

Study	Study Design	N	Age (years)	BMI	General Health	Intervention group (per day)	LC N-3 PUFA Dose g/d	EPA+DHA	Duration	Drop out	Lifestyle Modification	Outcomes
Ramel et al. [21]	Parallel	324	20-40	27.5 to 32.5kg/m²	healthy	A) 6 capsules + lean meat	A. 6mg/d	A. 0	8week	62	diet exercise	Endpoint leptin ↓ in C group (-22.9%)
						B) 150g cod (3 time/week)	B. 272mg/d	B. 54 + 207mg/d				D group tended to have ↑ endpoint ghrelin (5.6%)
						C) 150g salmon (3 time/ week)	C. 3004mg/d	C. 774 + 1370mg/d				weight loss (-5.2 ± 3.0 kg) in all the groups.
						D) Fish oil capsules + lean meat (6/ day)	D. 1418mg/d	D. 633+ 430mg/d				
Parra et al. [28]	Parallel	324	20-40	27.5 to 32.5kg/m²	healthy	A) 6 capsules + lean meat	A. 6mg/d	A. 0	8week	62	diet exercise	Decrease in BW in seafood group (C>B>D)
						B) 150g cod (3 time/week)	B. 272mg/d	B. 54 + 207mg/d				Oxidative stress ↓ based on MDA/ AOP ratio
						C) 150g salmon (3 time/ week)	C. 3004mg/d	C. 774 + 1370mg/d				
						D) Fish oil capsules + lean meat (6/ day)	D. 1418mg/d	D. 633+ 430mg/d				

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Ramel et al. [22]	Parallel	324	20-40	27.5 to 32.5kg/ m^2	healthy	A) 6 capsules + lean meat	A. 6 mg/d	A. ND	8week	62	diet exercise	weight loss (-5.2±3.2 kg P < 0.001) (C>D>B>A).
						B) 150 g cod (3 time/week)	B. 272mg/d	B. 54 + 207mg/d				↓ in SBP and DBP after the intervention (C>D>B). Lower baseline DHA % in erythrocytes was associated with greater DBP reductions.
						C) 150 g salmon (3 time/ week)	C. 3004mg/d	C. 774 + 1370mg/d				↓in WC (C>D>B).
						D) Fish oil capsules + lean meat (6/ day)	D. 1418mg/d	D. 633+ 430mg/d				
Wong et al. [37]	Parallel	27	18-75	≥ 30kg/ m^2	healthy	A) HC diet alone	4g/d	46%+38%	16 (12 I +4 M) week	2	diet	Significant reductions in BW BMI WC WHR BFM diastolic BP HOMA score in A & B groups
						B) HC diet + ω3 FAEEs						
Munro et al. [23]	Parallel	40	18-60	30 to 40kg/ m^2	healthy	A) 6 placebo capsules (n=14)	NR	70mg + 270mg	14 (4 I + 10 M) week	8	Diet	The mean weight loss higher in FO.
						B) 6 capsules of fish oil (n=18).						M phase further mean ↓ in weight in FO
Munro et al. [29]	Parallel	43	18-60	30 to 40kg/ m^2	healthy	A) 6 placebo (n=18)	NR	70 mg + 270 mg	12week	10	Diet	↓ in BW FM (B>A)
						B) 6 capsules of fish oil (n=15).						↑in FFM (B>A)
Hardena et al. [30]	Parallel	40	23-60	25.8 to 39.9kg/ m^2	healthy	70kcal 12 ml isocaloric 45% oil in water emulsion	2.8g/d DHA	NR	12week	19	Diet	No effect on BM and BCA in A group
						A) DHA						↓ in BW (A>B)
						B) placebo						
Ramel et al. [31]	Parallel	324	20-40	27.5 to 32.5kg/ m^2	healthy	A) 6 capsules + lean meat	A. 6mg/d	A. 0	8week	46	diet exercise	Physical activity and DHA ↓ the negative health effects associated with overweight
						B) 150g cod (3 time/week)	B. 272mg/d	B. 54 + 207mg/d				
						C) 150g salmon (3 time/ week)	C. 3004mg/d	C. 774 + 1370mg/d				
						D) Fish oil capsules + lean meat (6/ day)	D. 1418mg/d	D. 633+ 430mg/d				

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Neff et al. [32]	Parallel	49	18-65 years	25.0-40kg/m²	healthy	A) 5 mL/d of placebo	2g DHA	NR	*4.5 month	13	diet	plasma TG ↓ in group B
						B) 5 mL/d of algal DHA oil						TC ↑ in B group; no difference VLDL and LDL.
Cusson et al. [33]	crossover	25	>18	>25kg/m²	PCOS	A) 4 omega-3 capsules	4g/d	27 % + 56 %	8 I + 8 W # week	13	no	↓in fasting insulin BMI HOMA-IR levels in group A as compared to group B.
						B) 4 placebo						↓in TG [1.19 vs. 1.02 mmol/L] SBP [124.1 vs. 122.3 mm Hg] and DBP [73.2 vs. 69.7 mmHg] in A.
Crochemore et al. [34]	Parallel	45	60.83 (mean)	33 (mean)	post menopausal; T2DM	A) 2.5 g fish oil (n = 14)	A- 2.5g/d	A) 547.5mg + 352.5mg	4week	4	no	Group B with greater ↓ BW and WC glucose and TC and ↑ HDL.
						B) 1.5g fish oil (n = 14)	B- 1.5g/d	B) 328.5mg+ 211.5mg				
						C) placebo (n = 13).	C- 0g/d	C) 0mg				
Defina et al. [35]	parallel	128	30-60	26 to 39.9kg/m²	healthy	A) 5 placebo capsules (n=64)	3g/d	5:1 ratio	24week	41	diet and exercise (VO2 max)	↓ in BW BMI body fat and WC with no difference in group.
						B) 5 n- 3 capsules (n=64).						
Tapsell et al [36]	Parallel	126	18-60	25 to 37kg/m²	healthy	A) HC diet + placebo (n=42)	NR	420mg + 210mg	48week	62	diet and exercise	No difference in weight loss among all groups.
						B) HC fish diet + placebo (n=43).						
						C) HC fish Diet + LCn3PUFA supplements (n=41)						
Wong et al. [38]	Parallel	27	18-75	≥ 30kg/m²	healthy	C) HC diet alone	4g/d	46%+38%	16 (12 I +4 M) week	2	diet	changes in SBP HR plasma TGs C1 and C2 were significantly greater in B than A
						D) HC diet + ω3 FAEEs						
Gamme lmarka et al. [39]	Parallel	58	30-75	>25kg/m²		A) 2 g of fish oil	1.1g/d	640 + 480	6week	1	no	↑ in Serum adiponectin in A>B
						B) 2 g of placebo						

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Thorsdottir et al. [40]	Parallel	324	20-40	27.5 to 32.5kg/m²	healthy	A) 6 capsules + lean meat	A. 6mg/d	A. 0	8week	48	diet exercise	% EPA&DHA ↑ proportional to erythrocytes increase
						B) 150 g cod (3 time/week)	B. 272mg/d	B. 54 + 207mg/d				
						C) 150 g salmon (3 time/ week)	C. 3004mg/d	C. 774 + 1370mg/d				
							D. 1418mg/d	D. 633+ 430mg/d				
						D) Fish oil capsules + lean meat (6/ day)						
Moham madi et al. [41]	Parallel	64	20-35	25-40kg/m²	PCOS	A) 4 omega 3 capsules	4 g/d	720mg + 480mg	8week	3	no	↑ in adiponectin; ↓ glucose insulin HOMA-IR TC & LDL-C (A>B)
						B) 4 placebo						
Gravel et al. [42]	Crossover	170	18-65	22 to 32kg/m²	metabolic syndrome	A) Canola oil	A. 9.76 %	A. 0	40week	40	Diet	↑ in plasma adiponectin highest in B group
						B) Canola DHA	B. 2.30 %	B. 0				↓ in inflammatory biomarkers IL-6 & CRP
						C) Corn Saff	C. 10.27 %	C. 8.16 %				(B more significant)
						D) Flax Saff	D. 31.98 %	D. 0				
						E) Canola Oleic	E. 0.29 %	E. 0				
Kabir et al. [43]	Parallel	29	40-60	27-40kg/m²	post menopausal; T2DM	A) 3g placebo	1.8g/d	1.08g + .72g	8week	3	no	↓ in Adiposity TAG atherogenic and inflammatory biomarkers; no effect on IR
						B) 3g fish oil						
Parra et al. [44]	Parallel	324	20-40	27.5 to 32.5kg/m²	healthy	A) 6 capsules + lean meat	A. mg/d	A. 0	8week	62	diet exercise	↑ satiety with ↑ LC N-3 FA group (C> D> B)
						B) 150g cod (3 time/week)	B. 272mg/d	B. 54 + 207mg/d				
						C) 150g salmon (3 time/ week)	C. 3004mg/d	C. 774 + 1370mg/d				Thus positive relation B/W N-3 FA/n-6 FA ratio in fullness 2 h postprandial
							D. 1418mg/d	D. 633+ 430mg/d				
						D) Fish oil capsules + lean meat (6/ day)						

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Gunnars dottir et al. [45]	Parallel	324	20-40	27.5 to 32.5kg/ m^2	healthy	A) 6 capsules + lean meat	A. 6mg/d	A. 0	8week	62	diet exercise	↓TC by 0.2 mmol-1 greater C> B> D group.
						B) 150g cod (3 time/week)	B. 272mg/d	B. 54 + 207mg/d				
						C) 150g salmon (3 time/ week)	C. 3004mg/d	C. 774 + 1370mg/d				HDL ↓ greater in C > B
						D) Fish oil capsules + lean meat (6/ day)	D. 1418mg/d	D. 633+ 430mg/d				
Ninio et al. [46]	Parallel	75	25-65	≥ 25kg/ m^2	CVD: hypertension	A) fish oil	6g/d	0.36g + 1.56g	12week	25	diet exercise	B group showed ↑ HRV as compared with others.
						B) fish oil + exercise						↓in HR at rest and during sub maximal exercise IE in A and B
						C) sunflower seed oil						
						D) sunflower seed oil + exercise						
Ramel et al. [47]	Parallel	324	20-40	27.5 to 32.5kg/ m^2	healthy	A) 6 capsules + lean meat	A. 6mg/d	A. 0	8week	62	diet exercise	Weight loss (-5.2±3.2 kg).
						B) 150g cod (3 time/week)	B. 272mg/d	B. 54 + 207mg/d				↓inflammation parameters.
						C) 150g salmon (3 time/ week)	C. 3004mg/d	C. 774 + 1370mg/d				CRP= -32.0%; IL-6= -18.4%;
						D) Fish oil capsules + lean meat (6/ day)	D. 1418mg/d	D. 633+ 430mg/d				
Root et al. [48]	Parallel	57	18-30	>23kg/ m^2	healthy	A) 3 packet Omega 3	1.7g/d	690+ 1050mg	4week	6	no	↑ Production anti-inflammatory cytokines.
						B) 3packet Control						↓ in arterial stiffness and metabolic syndrome (A>B)
						*one packet consumed at breakfast two packets at dinner						
Ramel et al. [49]	Parallel	324	20-40	27.5 to 32.5kg/ m^2	healthy	A) 6 capsules + lean meat	A. 6mg/d	A. 0	8week	46	diet exercise	weight loss (-5.2 ± 3.0 kg) in all the groups.
						B) 150 g cod (3 time/week)	B. 272mg/d	B. 54 + 207mg/d				
						C) 150 g salmon (3 time/ week)	C. 3004mg/d	C. 774 + 1370mg/d				Fasting insulin & HOMA-IR ↓ in D
						D) Fish oil capsules + lean meat (6/ day)	D. 1418mg/d	D. 633+ 430mg/d				

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Rafraf et al. [50]	Parallel	64	20-35	25-40kg/m²	PCOS	A) 4 omega 3 capsules	4 g/d	720mg + 480mg	8week	3	no	No significant effect on BW BMI WC and WHR
						B) 4 placebo						↓ in glucose insulin HOMA-IR
Munro et al. [23]	Parallel	42	18-60	30 to 40kg/m²	healthy	A) 6 placebo (n=18)	NR	420mg + 1620mg	8 (4 I + 4 M) week	3	diet	Comparison among male and female is done and more effective loss in B W in female
						B) 6 capsules of fish oil (n=15).						
Bays et al. [51]	Parallel	167	18-79	25-43 kg/m²	hypertriglyceridemic	A) P-OM3 + fenofibrate 130mg	4g/d	NR	16week **	17 (RP); 33 (EP)	diet	No alteration BW or WC compared with baseline or compared with B group
						B) placebo + fenofibrate 130mg/d						
Sjoberg et al. [52]	Parallel	75	53 (mean)	31.7 (mean)	healthy	A) 6 Sunola oil (n=17)	A) 0	A) 0	12week	8	no	D group showed improvement in both LAC and HRV but no effect is seen on SAC and heart rate.
						B) 6 X 2g fish oil (n=16)	B) 2g;	B) 120mg + 520mg;				
						C) 6 X 4g fish oil (n=17)	C) 4g;	C) 240mg + 1040mg;				
						D) 6 X 6g fish oil (n=17)	D) 6g	D) 360mg + 1560mg				
Labonte et al. [53]	crossover	12	≥18	25-40kg/m²	type 2 diabetes	A) 5 × 1 g capsules fish oil	3g/d	NR	8week	nil	no	↓ in plasma TG in A>B
						B) control (5 × 1 g capsules						
Amarel et al. [54]	Parallel	12	20-40	27.5 to 32.5kg/m²	healthy	A) 6 capsules + lean meat	A. 6mg/d	A. 0	8week	nil	diet exercise	the methylation patterns slightly affected in B group
						B) 6 Fish oil capsules + lean meat	B. 1418 mg/d	B. 633+ 430mg/d				

NR-not report; N- sample size; PCOS-polycystic ovarian syndrome; T2DM- type 2 diabetes; I-intervention phase; M-maintenance phase; HC-hypo-caloric diet; BMI- Body mass index; EPA- Eicosapentaenoic acid; DHA- Docosahexaenoic acid; RP-randomised phase; EP- extension phase; W-washout phase; CRP-C-reactive protein; IL-6 -interleukin 6; BW- body weight; BCA- body composition analysis; HR- heart rate; WC- waist circumference; WHR- waist to hip ratio; HRV-heart rate variability; HOMA-IR- Homeostatic model assessment – insulin resistance; SBP- systolic blood pressure; DPB- diastolic blood pressure; C1- large artery elasticity; C2- small artery elasticity; HDL-high density lipoproteins; LDL- low density lipoproteins; VLDL- very low density lipoproteins; TC- total cholesterol; TAG- triacylglyceride.

*The study by Neff et al. the total intervention period is of 4.5 month in which for 21 days placebo was given to all 49 adults, later for 112 days they were randomised among placebo and fish group.

** The study by bays et al. the trial was run for 16 weeks (8 week RP) + 8 weeks EP). In RP the groups were randomized among the two groups. In EP, subjects received P-OM3 + fenofibrate. Subjects who continued with the same treatment in the extension phase are referred as non-switchers and those who initially received placebo + fenofibrate and later received P-OM3 + fenofibrate in the extension phase are switchers.

in study by Cusson et al. [30] participants went through a 32 week trial, with 8 weeks intervention and 8 week washout period.

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Discussion

Through various evidences it was found that intake of fish or fish oil capsules can decrease the weight of adult individuals with BMI ≥ 25 kg/m2. Among 31 studies there were 9 studies which did not provide any evidence for the biomarkers of intake [47-48] (DHA + EPA) that includes plasma concentration of EPA/DHA fatty acids, increases in the n3/n6 ratio of plasma FA [49], percentage of N 3 FA in serum phospholipids and the ratio of n6: n3 platelet membrane phospholipids etc. These biomarkers play a key role in achieving the proposed outcomes of a study, an alteration in any of them act as confounding factor or/and may create a bias in the study [27,29,30, 36,42,47,50]. Review consist of studies in which participants have went through some or the other kind of dietary and physical activity modification which are judged by FFQ, 3: day diary record, 24hr dietary recall etc under free living conditions that cause attrition in the negative outcomes. As such cases, participants tend to record some false details that may induce a bias [21,35-36].

There is conflicting evidence on the effect of LC N:3 PUFA on weight loss found in the review articles. Du et al. [51] did a review on the anti: obesity effect of fish oil, in which 21 studies were included. Through meta: analysis, they concluded no association between fish oil and weight loss. On the other hand, Buckley et al. [52-53] who published a review article in 2009:2010 [52-53] with the same objective, but was not based on meta: analysis. In this review, no evidences were reported which would conclude the beneficial effect of fish oil in obese individual.

Unlike the reviews mentioned above, a meta: analysis was done by Bender et al. [54] in 2014 on 12 RCT's. A significant difference was estimated among the intervention and control group in terms of weight loss (P=0.047), waist circumference (P=0.003) and body fat. Rather they stated a beneficial role of long term intervention of fish oil on weight loss (P >0.017) [47].

In context of the adiponectin level which is. referred as obesity bio marker, the current review found a positive correlation between LC N:3 PUFA and serum adiponectin concentration. On the contrary however, a review conducted by Gray et al. [55] on 11 clinical trials and observational studies showed otherwise. Through their review they concluded with the potential role of omega 3 in weight maintenance when given with a calorie restricted diet but no overall effect on adiponectin levels. In contradiction to the review by Gray et al. [55] & Wu et al. [56] published a meta: analysis on 14 RCT's in 2013 for analysing the effect of DHA + EPA on adiponectin levels. According to this, a potential increase in the circulating adiponectin levels were found in the group supplemented with omega 3 (P= 0.02) [56].

In the present review, there were several studies which were in agreement of the hypothesis while other was not. All the studies were based on intention to treat analysis, which has its own drawback, because in this case withdrawals and deviations are usually ignored, leading to biasing in the results. The study by Ramel et al. [21] which was a SEAFOO plus YOUNG study included in this review, demonstrated all the objective of the review in the form of positive results [21,25,27,41,43,50].

On the other hand, Gammelmark et al. [33] failed to support the role of omega 3 in weight loss and another objective. As the studies included in the reviews were conducted for shorter duration with low dosage. A huge variation in the supplemented value was observed in the review studies ranging from 1:6g of omega 3/day. There are 6 studies in which WHO standards of recommendation [57] have not been followed like in the study by Crochemore et al. [31], Kabir et al. [39], Root et al. [44], Hardena et al. [28], Gammelmark et al. [33], Neff et al. [29].

In the present study, some indications were found that the effect might be greater in men than in women for the outcome waist circumference, which is a measure for visceral adiposity. This is relevant, as visceral fat is strongly associated with metabolic disease risks [21,45]. Several studies stated that n3: PUFA had a greater effect on weight loss in men than women [58], whereas other studies found stronger effects in women [26]. Difference among the sexes in the physiological response to omega: 3 is plausible because men and women have a different fat tissue anatomy and physiology.

In evidence of some animal studies, supplementation with DHA+EPA is effective in reducing weight and fat mass in mice [58] while others have shown no significant effect [59,60]. Studies involving human subjects have also stated the conflicting results, as found in present review. In reference of the positive outcome, a small number of severely obese women following a Very Low-density lipoproteins reported a significant weight loss in the group that also consumed LC N:3PUFA (2·8g/day fish oil; EPA: DHA = 2:1) compared to the control [61].

Reduced levels of CRP, a biomarker of CVD risk, are directly related to a reduction in weight loss. However, in study by Ramel et al. [43], there was a 2: fold greater decrease in CRP levels for group consuming highest percentage of omega: 3 during the weight loss phase as compared to placebo group, and CRP continued to decrease during the maintenance phase also. This would suggest that a greater decrease in CRP for FO could be attributed to LCN:3PUFA [62]. There were some studies mentioning or reporting conflicting findings, with one study stating that DHA+EPA intake but not weight loss was associated with a significant reduction in CRP [63], on the other hand, Madsen et al. [64] reported that LC N:3 PUFA had no effect on serum concentrations of CRP [64]. Differences in the sample size and age of the two groups and the length of the intervention could account for the conflicting results. Because of the current limited information, it is difficult to completely explain why fish oil decreased abdominal fat.

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Unlike previous reviews [51,64], which have intervention period of not more than 6 months whereas in our review included trials were of longer duration maximum up to 1 year. In addition to this, among all the studies the highest dose of 6 g of LC N:3 PUFA/ day was also given to seek for the positive effect [65-71].

Limitations

The included parallel and crossover trials had many differences including subject health status, nutritional therapy, physical activity modification, fish oil supplement doses (1:6g/ day) and compositions (EPA: DHA), follow: up durations, and treatment methods (parallel single blind or double blind/ crossover trial). Secondly, the comparison was made within trials with wide range of difference in treatment durations (4 to 48 weeks). Moreover, the meta: analysis method of analysis was not used in this review. Also, only 2 electronic databases were used for searching articles, in comparison to other reviews that have used Medline, Embase, Cochrane Library and many others for getting relevant records. This might be an important reason masking why the anti: obesity effects of fish oil supplementation were not as obvious in humans as animals.

Conclusion

Omega:3 fatty acid supplementation especially DHA & EPA has been widely utilised for a variety of medical conditions that includes CVD, hypertension, T2DM and arthritis. Till date, results of studies investigating their impact on obesity development and progression of disease are not clear. In conclusion, from the results of our review, no effective proof was found to say that LC N:3 PUFA intake can definitely decrease body weight in overweight/obese adults.

Although, the majority of studies included in our review did not concluded the same in their findings. Besides, only few studies stated the potential role of LC N:3 PUFA in attenuating the weight gain in maintenance phase when accompanied with lifestyle modifications (energy restriction and raising physical activity level). On the other hand, some preliminary evidence was founded that an increase in LC N:3 PUFA intake within same dose range might reduce the postprandial sensations of hunger, which helps in weight management. Data from a number of studies also suggest that LC N:3 PUFA might promote increases in plasma HDL levels and heart rate variability thus potentially increasing the elasticity in large: small arteries and indirectly assisting with CVD risk reduction. It is also possible that the increases in plasma omega 3 concentrations, have a beneficial effect in reducing the blood glucose, HOMA:IR levels in type 2 diabetic patients even at lower dose levels (1.5g/day).

While there is growing evidence that LC N:3 PUFA can improve body composition in humans, there is still much of contradiction as the majority of studies are of relatively short duration. Accordingly, there is an urgent need for longer: term studies and meta-analysis with appropriate dosage recommendation as per WHO i.e. 2:4g/day to determine the result of long chain omega:3 supplementation on body weight in overweight and obese populations. Moreover, a proper assessment of parameters like plasma concentration of EPA/ DHA fatty acids, increases in the n3/n6 ratio of plasma FA and percentage of N3 FA in serum phospholipids should be included for effective study of proposed outcome. This would help in getting a definite conclusion.

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