



REVIEWS: CURRENT TOPICS

Omega-3 fatty acids in obesity and metabolic syndrome: a mechanistic update[☆]

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Abstract

Strategies to reduce obesity have become public health priorities as the prevalence of obesity has risen in the United States and around the world. While the anti-inflammatory and hypotriglyceridemic properties of long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) are well known, their antiobesity effects and efficacy against metabolic syndrome, especially in humans, are still under debate. In animal models, evidence consistently suggests a role for n-3 PUFAs in reducing fat mass, particularly in the retroperitoneal and epididymal regions. In humans, however, published research suggests that though n-3 PUFAs may not aid weight loss, they may attenuate further weight gain and could be useful in the diet or as a supplement to help maintain weight loss. Proposed mechanisms by which n-3 PUFAs may work to improve body composition and counteract obesity-related metabolic changes include modulating lipid metabolism; regulating adipokines, such as adiponectin and leptin; alleviating adipose tissue inflammation; promoting adipogenesis and altering epigenetic mechanisms.

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Keywords: Adipocytes; Fish oil; Metabolic syndrome; Obesity; Omega-3 polyunsaturated fatty acids; Weight loss

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Abbreviations: ALA, α-linolenic acid; AA, arachidonic acid; BMI, body mass index; BAT, brown adipose tissue; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FABP, fatty acid-binding protein; FFAR, free fatty acid receptor family; FGF, fibroblast growth factor; HDL, high-density lipoprotein; HF, high-fat; IL, interleukin; LA, linoleic acid; MetS, metabolic syndrome; MUFA, monounsaturated fatty acid; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TG, triglycerides; T2DM, type 2 diabetes mellitus; UCP, uncoupling protein; VLDL, very low density lipoprotein; WAT, white adipose tissue.

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1. Introduction

The American Medical Association recognizes obesity as a disease [1] and considers it a major public health problem. In the United States, 36.5% of adults are obese [2], while approximately 39% of the world's adult population is overweight and more than 13% are obese [3]. Obesity increases morbidity risks for heart disease, type 2 diabetes mellitus (T2DM) and some types of cancer [4]. Metabolic changes associated with these diseases comprise metabolic syndrome (MetS), which is diagnosed when three of the following five conditions exist: abdominal obesity, elevated triglycerides (TG), reduced high-density lipoprotein (HDL) cholesterol, high blood pressure and elevated fasting blood glucose [5].

Lipids are key macronutrients in the human diet. The type and proportion of dietary fatty acids consumed impact health and whole-body physiology [6]. Research has shown that saturated fatty acids (SFAs) are detrimental to health, while monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) offer health benefits [7]. In the diet, fatty fish and fish oil rich in omega-3 (n-3) PUFAs, such as eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), have demonstrated cardioprotective, anti-inflammatory and hypotriglyceridemic properties. Hence, these fatty acids may assist in the treatment and prevention of obesity comorbidities, especially by improving individual components of the metabolic syndrome [7–9]. Therefore, the effect of n-3 PUFAs on body weight and body composition is of particular interest.

In this review, we provide an update on the effects of n-3 PUFAs on obesity and MetS in both animal and human studies, highlighting potential mechanisms for n-3 PUFAs in reducing body weight, improving body composition and counteracting the adverse metabolic consequences of obesity.

2. Adipose tissue and obesity

Body fat is primarily stored in adipose tissue, a connective tissue composed of adipocytes, preadipocytes, vascular endothelial cells, fibroblasts and various types of immune cells, including adipose tissue macrophages [10]. Adipose tissue is an active endocrine organ that secretes numerous hormones, including leptin and adiponectin, and cytokines (adipokines) such as interleukin (IL)-6 [11]. Three major types of adipose tissue have been identified: white adipose tissue (WAT), brown adipose tissue (BAT) and beige (“brite”) adipose tissue. WAT is primarily responsible for energy storage in the form of TG and the release of fatty acids during periods of fasting; it is mainly located in two distinct depots, as subcutaneous adipose tissue or visceral adipose tissue [10]. The adipocytes of visceral fat surrounding internal

organs are more metabolically active than those of subcutaneous adipose tissue and thus contribute to the risks of cardiovascular disease and T2DM [12]. BAT plays a key role in thermogenesis and is mainly found above the clavicle and scapula in adults [12]. Obesity leads to adipose tissue dysfunction, which is mechanistically linked to the pathogenesis of insulin resistance in the liver and in skeletal muscle (Fig. 1) and may result in MetS [13].

While weight loss via lifestyle modification is the primary treatment in the management of obesity and its comorbidities, compliance is difficult. Adjunct treatments for the management of obesity include pharmaceuticals [14], surgery [15] and dietary supplements [16]. Despite these measures, however, the prevalence of obesity has continued to rise. Thus, alternative strategies to assist in weight loss and reduce body fat are necessary. Natural bioactives such as n-3 PUFAs present few side effects and so may be safer than other modalities for the treatment of obesity. This review summarizes current basic and clinical research and mechanistic insights regarding the effects of n-3 PUFAs on obesity.

3. Omega-3 fatty acids

3.1. Synthesis and metabolism

The human body can synthesize many fatty acids, but not linoleic acid (LA; omega-6; C18:2 n-6) or α -linolenic acid (ALA; C18:3 n-3), which must be consumed in the diet. ALA is the precursor for EPA (C20:5 n-3), docosapentaenoic acid (DPA; C22:5 n-3) and DHA (C22:6 n-3) in the human body (Fig. 2) [6]. Many studies have found low conversion rates of ALA to EPA and DPA, and little to no DHA synthesis [17,18]; hence, any direct benefits of these very long chain fatty acids depend on dietary intake [17]. Both dietary intake and fatty acid desaturase activity determine plasma n-3 PUFA levels [19]. A balanced n-6:n-3 fatty acid ratio (1:1 to 2:1 is optimal) is important for homeostasis and normal development throughout the lifespan. High n-6 PUFA intake in the Western diet increases the n-6:n-3 ratio to a range from 10:1 to 20:1 and may play a role in the pathogenesis of obesity and related diseases [19,20].

3.2. Sources of omega-3 PUFAs

Dietary sources of n-3 PUFAs are much less abundant than n-6 PUFAs. ALA is synthesized by plants from LA and can thus be found in green leafy vegetables, seeds such as flaxseed (linseed), nuts and legumes. Vegetable oils such as sunflower, corn, perilla, canola and soybean also provide ALA but are much more abundant in LA. Fish, such as salmon, tuna, trout, mackerel, anchovy, bluefish, herring,

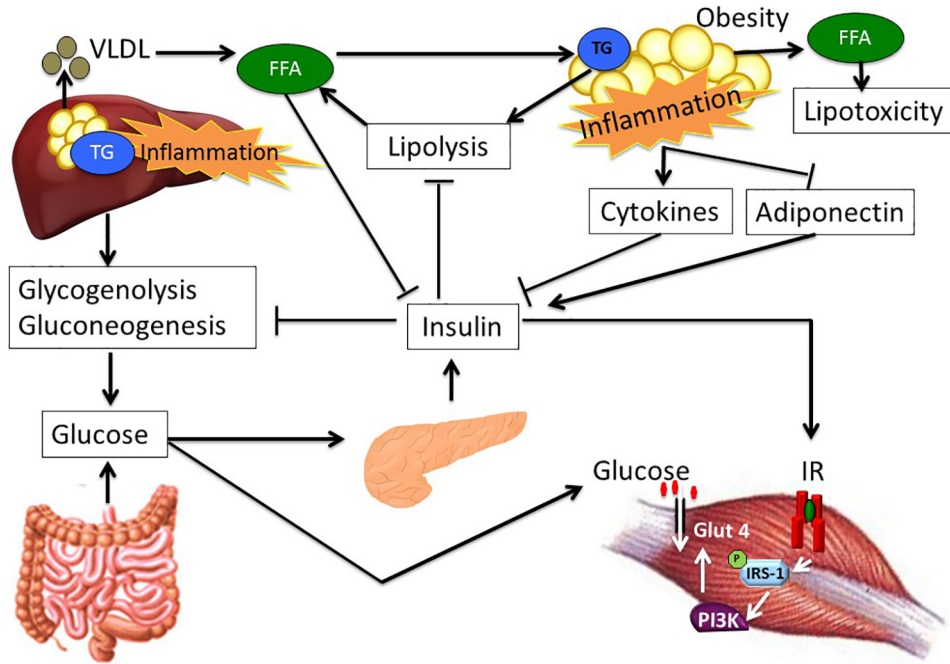


Fig. 1. Adipose tissue, liver and skeletal muscle cross talk in obesity and insulin resistance. The liver maintains normoglycemia during fasting via glycogenolysis and gluconeogenesis. Following a meal, increased glucose delivery to the pancreas stimulates insulin secretion, which acts on the liver, adipose tissue and skeletal muscle. The primary action of insulin on the liver is to suppress hepatic glucose output, while insulin increases glucose uptake by the skeletal muscle and adipose tissue. Insulin additionally inhibits lipolysis in adipose tissue. In obesity, changes in adipokines produced and released from adipose tissue, such as decreased adiponectin and increased TNF- α and other inflammatory cytokines, coupled with increased free fatty acids contribute to hepatic and skeletal muscle insulin resistance.

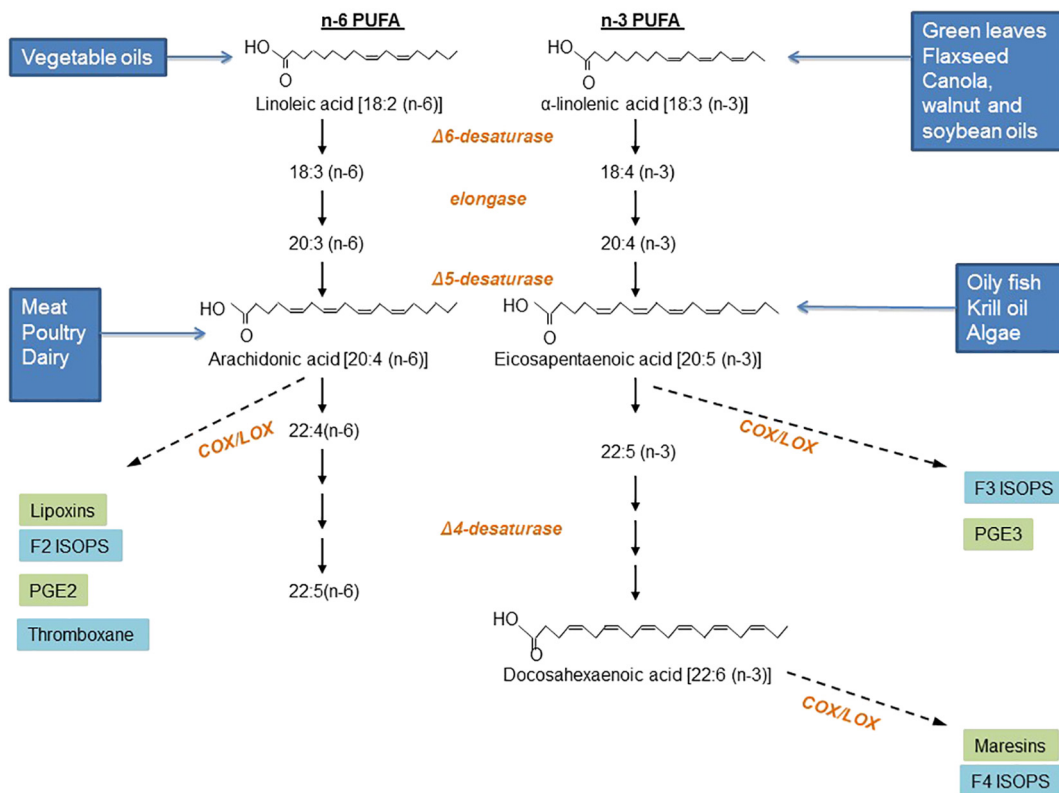


Fig. 2. Metabolism of omega-6 and omega-3 polyunsaturated fatty acids. LA is an essential n-6 PUFA that is metabolized to AA and further to proinflammatory eicosanoids. ALA is an essential n-3 PUFA that is metabolized to EPA, DPA and DHA. Eicosanoids derived from the metabolism of EPA, DPA and DHA also aid in the regulation of inflammation and are considered more anti-inflammatory. ISOPS, isoprostanes; COX, cyclooxygenases; LOX, lipoxygenases.

mullet, sturgeon and sardines, are also rich sources of n-3 PUFAs, particularly EPA and DHA. Lean fish, such as cod, store lipids in their liver, and for this reason, cod liver oil is a good source of n-3 PUFAs. Fatty fish, such as salmon, mackerel, sardines and tuna, store lipids throughout their bodies and are good whole sources of n-3 PUFAs [6].

The 2015 Dietary Guidelines for Americans recommend consuming about 8 oz per week of seafood, which would provide about 250 mg/day of EPA and DHA [21]. The recommended intake for n-3 PUFAs corresponds to consuming fish twice weekly, including one serving of oily fish. Even though there is ample evidence for a role of n-3 PUFAs in modulating chronic diseases, an optimal dose has not been agreed upon, and recommendations vary based on governing body. The U.S. Food and Drug Administration has stated that levels up to 3 g/day are generally recognized as safe [22], although other authorities have reported no adverse effects at up to 5–6 g/day [23]. It has been suggested that the bioavailability of n-3 PUFAs is improved by emulsification. Emulsified n-3 PUFA is more easily exposed to pancreatic lipase and colipase, enhancing its digestion. Additionally, emulsified n-3 PUFA is easily transported into enterocytes, thus increasing fatty acid absorption [24,25].

3.3. Dietary omega-3 PUFA intake, obesity and metabolic disorders

Dietary fish intake is considerably higher in people of the circumpolar arctic regions and relatively much lower in those living in the United States, Australia, France and the United Kingdom. Fish intake closely reflects n-3 PUFA consumption, with intakes of approximately 3 to 4 g/day by Eskimos, 5 to 6 g/day by Japanese, 0.189 g/day by Australians and 0.25 g/day by Europeans and North Americans [26,27]. After it was reported that Japanese and Eskimo populations had healthier metabolic profiles associated with elevated plasma n-3 PUFA levels attributed to high fatty fish intake [28], many prospective studies began to examine whether fish or fish oil intake prevents the development of obesity.

The Health Professional Follow-up Study suggested that men with a high level of fish consumption were less likely to be overweight [29]. In contrast, the Nurses' Health Study found that women with higher fish intake (two or more fish meals per week) had a higher risk of being overweight [30]. In China, data from the Shanghai Women's and Men's Health studies found similar indices of body mass among groups of varying fish intake [31]. Clearly, findings of prospective studies regarding the beneficial effects of fish intake on obesity are far from agreement. The evident discrepancies may have arisen due to differing or inadequate methods of data collection on fish intake (food frequency questionnaires), differences in cooking methods and other unaccounted for lifestyle practices (exercise, etc.) from study to study and among different study populations.

Plasma, erythrocyte and tissue n-3 PUFA concentrations are largely determined by consumption and thus may be taken to accurately reflect n-3 PUFA consumption. Plasma levels of fatty acids reflect recent intake, whereas tissue levels of fatty acids reflect long-term intake [32,33]. Erythrocyte fatty acid content (i.e., the omega-3 index) correlates with fatty acid intake and parallels tissue concentrations and thus is more reflective of long-term intake [34]. An increase in n-6:n-3 ratio [35] and overall lower serum phospholipid n-3 concentrations, particularly of DHA have been associated with obesity, specifically waist circumference measures [36] in obese adolescents [35] and obese adults [36]. Thus, prospective studies on the relationship between plasma and erythrocyte fatty acid content and the long-term risk of obesity are warranted to clarify this issue.

4. Omega-3 PUFA in animal studies

Animal studies performed to investigate the antiobesity effects of n-3 PUFA have used a variety of models, diet compositions, and n-3

PUFA compositions and doses. Such differences across experimental designs complicate the interpretation of their results into cohesive and conclusive findings (Table 1).

4.1. Omega-3 PUFA, energy intake and obesity

Studies relating effects on body weight and energy intake with n-3 PUFA supplementation are inconsistent; while some show either decreased [37,38] or increased energy intake [39], most show unchanged energy intake with the addition of n-3 PUFA or with varying n-3 PUFA doses [40–57]. Only one study performed with female mice reported decreased energy intake with no significant effect on body weight [38].

Supplementation with n-3 PUFA prevented high-fat (HF)-diet-induced weight gain in a number of rodent studies, most of which supplemented an HF diet provided from the start, concurrent with inducing obesity [41,43,45,48,49,51]. Several studies have utilized a design that investigated the effectiveness of n-3 PUFA to reverse diet-induced weight gain and related metabolic changes by adding n-3 PUFA to the HF diet at approximately midstudy [46,47,58,59]. Our lab found that mice on an EPA reversal diet (6 weeks of HF followed by 5 weeks of HF-EPA) had body weights similar to mice fed the HF-only diet [47]. In a different study, body weight decreased significantly at the beginning of the 6-week reversal period, returning to weights similar to the low-fat-fed group for the remainder of the study (18 weeks total) [58]. Taken together, these studies suggest that antiobesity effects of N-3 PUFA in mice are predominantly seen when it is fed from the start rather than introduced after obesity is already established.

4.2. Omega-3 PUFA and insulin resistance

Obesity leads to insulin resistance, which is at least in part responsible for the pathogenesis of MetS [13]. Most weight loss interventions improve insulin resistance. Similarly, most animal studies document a beneficial effect of n-3 PUFAs on insulin sensitivity [60]. Since n-3 PUFAs induce weight loss in rodent models of obesity, it is difficult to state whether there are direct effects of n-3 PUFAs on insulin sensitivity. By contrast, we have shown weight-independent benefits of EPA on insulin sensitivity in HF-diet-induced obese C57BL/6J mice. These EPA-fed mice had significantly improved homeostatic model assessment of insulin resistance (HOMA-IR) scores when compared to HF-fed mice, despite similar body weights [47].

4.3. Animal study conclusions

Thus far, most rodent studies have shown an antiobesity effect of n-3 PUFA, while fewer studies have found no change in body weight [37,39–41,44–46,49,51–57,59,61,62]. These studies do suggest that n-3 PUFA plays a role in reducing adipose tissue mass [40], particularly in the epididymal [39,42,43,46,49,52,53,56,57,61–64] and retroperitoneal locations [37,39,41,46,49,57]. Differences in the outcomes of studies on the effects of n-3 PUFA on body weight could be due to differing animal models of obesity (genetic vs. diet-induced obesity), the content of the diet (HF vs. high sucrose), the n-3 PUFA (EPA or DHA) formulation, the form of n-3 PUFA (TG form or as ethyl ester) provided or various combinations of these factors. Differences in n-3 PUFA dosage and duration may contribute to differences in outcomes as well. Failure to assess energy expenditure also limits meaningful comparisons. The combination of calorie restriction and n-3 PUFA supplementation may be the most effective strategy for reducing weight and improving body composition [54]. Interestingly, Ruzickova et al. extrapolated findings from their animal study to humans to suggest that with a daily intake of 100 g dietary fat, 11 g of EPA/DHA would be required to limit weight gain [42]. Few animal studies have

Table 1
Effects of n-3 PUFA on body weight and body composition in male animals

Rats/mice (animal number/days on diet)	Dietary fat (% kcal from fat)	Saturated fat (SFA) (%)	n-3 PUFA (% in diet)/replaced/added	n-6/n-3 ratio	Control diet	Body weight	Fat mass/site	Ref.
OETF rats (8/175)	5	NA	EPA Added	NA	Safflower oil	NC	—/ABD	[40]
Wistar rats (30/28)	20	NA	0.22–5.48 EPA 0.00–5.38 DHA	NA	Lard and olive oil	NC	—/RP	[41]
C57BL/6J mice (8/49)	20	1.86	6.5 n-3 PUFA 0.60 EPA 5.40 DHA	1.08	Corn oil	—	—/EPI	[42]
	20	2.12	Replaced 44% 5.94 n-3 PUFA 4.26 EPA 0.74 DHA	1.04	Corn oil	—	NC	
	20	1.4	Replaced 15% 12.48 n-3 PUFA 0.72 EPA 1.60 DHA	0.21	Flaxseed oil	NC	NC	
	20	1.14	Replaced 15% 14.14 n-3 PUFA 2.12 EPA 4.72 DHA	0.14	Flaxseed oil	—	—/EPI	
	35.2	6.79	Replaced 44% 5.56–12.70 n-3 PUFA 0.40–1.16 EPA 3.60–10.28 DHA	1.15	Rapeseed oil, sunflower oil	—	—/EPI	
C57BL/6J mice (12/35)	20	1.14	Replaced 15%–44% 14.14 n-3 PUFA 2.12 EPA 4.72 DHA	0.14	Flaxseed oil	—	—/EPI	[43]
	35	6.79	Replaced 44% 5.56 n-3 PUFA 0.40 EPA 3.60 DHA	1.15	Chow fed	—	—/EPI	
Wistar rats (29/35)	62		Replaced 15% 0.10 EPA Added		SFA	NC	—/RP	[37]
21-week sucrose-induced obese Wistar rats (10/42)	7.5	2.7	2.7 n-3 PUFA 1.5 EPA 0.98 DHA	0.02	Corn-canola oil	NC	NC/EPI	[59]
<i>fa/fa</i> and lean Zucker rats (8/63)	10	2.542–2.624	NA .0089–3.55 ALA 0.80 EPA 0.161 DPA 0.915 DHA	0.54–58.59	Flaxseed oil Menhaden oil or Safflower oil	NC	NC	[44]
C57BL/6J OlaHsd mice (30/182)	40	9.6	NA 21.9 n-3	7.5	HF	NC	NC	[45]
Sprague-Dawley rats (48/21)	14	5.24	NA 3.42 n-3 0.21 EPA 1.08 DHA	0.2	Sucrose diet	NC	—/RP and EPI	[39]
Wistar rats (8/270)	8	1.65	NA 1.66 n-3 Replaced 7%	2.30	Cornstarch	NC	—/RP and EPI	[46]
C57BL/6J mice (10/77)	45	13.5	Added 7.25 EPA (6.75)		HF diet	—	—	[47]
C57BL/6J mice (14/70)	45	NA	Added 3.6 EPA (also says 36g/kg)	NA	HF diet	—	—/SubQ PG MES	[48]
Golden Syrian hamsters (12/140)	45	NA	Added 2.0 n-3 0.9 EPA 0.5 DHA	3.75	HF lard diet	—	NC	[154]
Wistar rats (NA/16-20)	50	NA	Replaced 10% FO Replaced 30%	NA	HF lard diet	NC	—/SubQ RP EPI	[49]
C57BL/6J Ob/ob (NA/112)	NA	NA	Cod liver oil 80 g EPA 80 g DHA	NA	Primrose oil	—	NC	[50]
C57BL/6J mice (7/140)	38.1	NA	NA 5% EPA ethyl ester Replaced 5%	NA	HF diet	NC	NC/WAT	[51]
	25	NA	Added 5% EPA ethyl ester Replaced 5%	NA	HF-HS diet	—	—/WAT	
C57BL/6J mice (8/213)	60	NA	Added 5.3 n-3 PUFA 0.74 EPA 2.4 DHA	NA	cHF diet	NC	—/EPI	[52]

(continued on next page)

Table 1 (continued)

Rats/mice (animal number/days on diet)	Dietary fat (% kcal from fat)	Saturated fat (SFA) (%)	n-3 PUFA (% in diet)/replaced/added	n-6/n-3 ratio	Control diet	Body weight	Fat mass/site	Ref.
Wistar rats (16/28)	NA	NA	Replaced 15% NA	NA	HF-HS diet	NC	–/EPI	[53]
C57BL/6J mice (50/49)	35	NA	Replaced 6% 0.42 n-3 PUFA 0.19 DHA 0.05 EPA	NA	HF diet Corn oil	NC	–/BAT	[54]
Sprague–Dawley rats (28/21)	20	9.4	Replaced 15% 20 n-3 PUFA 2 EPA 6.4 DHA	NA	Safflower	NC	–/EPI PER	[61]
C57BL/6J mice (8/49)	35	NA	0.5 EPA+DHA Replaced 10%	NA	Corn oil	–	–/EPI	[63]
Goto–Kakizaki rats (15/28)	NA	NA	0.5 g/kg EPA Gavage	NA	CMC	NC	NC	[55]
KKA ^y mice (36/84)	10	NA	12 EPA 18 DHA Added 30 %	NA	(1) Perilla or (2) Soybean oil & (3) Lard	NC	–/EPI	[56]
Fisher rats (10/42)	20	NA	18 n-3 PUFA	NA	Corn oil	NA	–/EPI	[64]
Wistar rats (28/16)	50	9	5% n-3 PUFA 2.62 EPA & 1.62	0.4	Lard	NC	–/EPI	[62]
Wistar rats (30/24)	40	23.7	Replaced 10% 18-6 or 40.6 n-3 PUFA 5 or 17.9 EPA 7.7 or 14.7 DHA	0.29 or 0.12	Olive oil + Beef tallow	NC	–/EPI & RP	[57]

+, increase; –, decrease; A, addition; ABD, abdominal; CMC, carboxymethylcellulose; EPI, epididymal; FO, fish oil; HF-HS, high fat and high sugar; MES, mesenteric; NA, information not available; NC, no change; OTLEF, Otsuka Long–Evans Tokushima fatty; PG, perigonadal; PER, perirenal; RP, retroperitoneal adipose tissue; R, reversal; SubQ, subcutaneous.

considered translation to human studies since the amounts of n-3 PUFA, as EPA, DHA or both, in animal studies far exceed amounts feasible in humans [42,45,64]. It should be noted, however, that human studies of fish oil intake among Eskimo and Japanese populations have shown beneficial effects of these fatty acids even at intake levels below 11 g/day. Since these populations consume more fish and less red meat, it is plausible that a relatively lower

arachidonic acid (AA) intake leading to a decreased n-6:n-3 ratio is contributing to the beneficial effects observed.

5. Omega-3 PUFA and weight loss in humans

There are a variety of approaches to investigating the effects of n-3 PUFA on body weight, body composition and energy intake in human

Table 2
Effects of n-3 PUFA on body weight, body composition and energy intake in humans

N (M/F)	Participants Age (years)	n-3 PUFA (% in diet)	Other diet	Duration	Body weight	Fat mass	Fat site	Lean mass	Energy intake	Ref.
15 (10/5)	Healthy adults 69–73 years	4 g Lovaza/day 1.86 g EPA 1.50 g DHA	Corn oil NA	8 weeks	NC	NC	NA	NC	NA	[186]
44 (15/29)	Healthy adults 62–76 years	4 g n-3 1.86 g EPA 1.5 g DHA	Corn oil	6 months	NC	NC	NA	+	NA	[210]
17 (8/9)	Nonobese, T2DM Obese, T2DM 41–66 years	PUFA diet NA	SF diet	10 weeks 5 week crossover design	NC	–	Abdominal SubQ	NA	NC	[71]
33 (20/13)	Overweight, impaired glucose 40–61 years	3.9 g EPA + DHA	Maize oil	9 months	NC	NC	NA	NC	NA	[187]
53 (17/36)	Decreased muscle mass 60+ years	1.3 g n-3 660 mg EPA 440 mg DHA	Vitamin E	12 weeks	NC	NC	NA	NC	NA	[211]
76	Overweight, IR 9–18 years	540 mg EPA 360 mg DHA	Corn starch	1 month	NC	NC	NA	NA	NA	[126]
126 F	Healthy, postmenopausal 68–82 years	1.2 g EPA + DHA	Olive oil	6 months	NC	NA	NA	NA	NC	[70]
44 (14/30)	Healthy 27–41 years	1.6 g EPA 800 mg DHA	Safflower oil	6 weeks	NC	–	NA	+	NA	[66]
12 F	Postmenopausal, T2DM, without hypertriglyceridemia 54–56 years	1.8 g n-3 1.08 EPA 0.72 DHA	Placebo Paraffin oil	2 months	NC	–	SubQ	NA	NC	[69]
47 M	Overweight 35–55 years	230 mg EPA and 154 mg DHA	Corn oil	8 weeks	NC	NA	NA	NA	NC	[68]
66 (45/21)	T2DM 40–70 years	2.8 g EPA + DHA	Placebo	24 weeks	NC	NA	NA	NA	NA	[208]
24 M	Viscerally Obese	1.8 g EPA + 1.56g EPA	Placebo	6 weeks	NC	NA	NA	NA	NA	[212]
27F	Overweight/obese 23–60 years	2.8 g DHA	Oleic acid	12 weeks	NC	NC	NA	NC	–	[67]

F, female; IR, insulin resistant; M, male.

Table 3
Effects of n-3 PUFA in addition to dietary intervention on body weight, body composition and energy intake in humans

N (M/F)	Participants	Diet intervention	Dietary fat (%)	n-3 PUFA (% in diet)	Control (placebo)	Duration	Body weight	Fat mass	Lean mass	Energy intake	Ref.
P18 (6/12) 15 (5/10)	Obese 28–53 years	HEWLD	NA	6 g n-3 0.42 g EPA 1.62 g DHA	MUFA	12 weeks	NC	NC	NA	NC	[213]
24 (6/18)	Obese 25–65 years	LCD	25	1.8 g n-3 DHA:EPA 5:1	Placebo Corn oil	12 weeks	NC	NC	NA	NA	[73]
35 F	Overweight, IR 21–69 years	Low fat/high carb diet	35	1.3 g EPA 2.9 g DHA	2.8 g LA 1.4 g OA	24 weeks	NC	NC	NC	NA	[120]
6(5/1)	Healthy 21–25 years	Controlled diet	32	Replaced 6g: 6 g FO 1.1 g EPA 0.7 g DHA	None	3 weeks	NC	—	NC	NC	[65]
14 (8/6)	Overweight, hypertensive 51–55 years	CR + daily fish meal	NA	3.65 g n-3	CR only	16 weeks	NC	NC	NA	NC	[75]
35 M	Overweight 20–40 years	30% CR + cod	NA	0.3 g n-3	CR only	8 weeks	—	NA	NA	NC	[76]
42 M	Overweight 20–40 years	30% CR + salmon	NA	3.0 g n-3	CR only	8 weeks	—	NA	NA	NC	
29M	Overweight 20–40 years	30% CR + FO	NA	1.5 g n-3	CR only	8 weeks	—	NA	NA	NC	
34 M	Overweight 25–65 years	Ketogenic Mediterranean	45.8 ± 4	Krill oil 57.5 mg EPA 32.5 mg DHA	Ketogenic Mediterranean diet only	4 weeks	NC	NC	NC	NA	[74]
45	MetS 37–63 years	CR+ FO	27.7	2.13 G N-3 PUFA	CR	12 weeks	—	NC	NC	NC	[72]
278	Overweight 20–40 years	30% CR	Lean fish or fatty fish	0.26 g in lean fish & 2.1 g in fatty fish	CR + placebo	8 weeks	NC	NC	NC	NC	[214]
51 F	Healthy 20–50 years	30% CR	Fish oil 30	1.3 g EPA and DHA 1.3 g EPA	Placebo Sunflower flower CR only	10 weeks	NC NC	NC NC	NC NC	NC NC	[118]

CR, calorie restriction; HEWLD, healthy eating weight loss diet; LCD, low-calorie diet; MetH, metabolically healthy; OA, oleic acid.

interventions that use different types of fish and varying levels of fish oil content, particularly EPA and DHA (Table 2). Fish and fish oil have also been used in addition to a variety of weight loss and dietary interventions of different durations with or without an exercise regimen. Participants have ranged from healthy to obese, with a variety of obesity-associated disorders, including T2DM, hyperinsulinemia and other features of MetS. The control, or type of placebo, which consists of assorted oils containing n-6 PUFA, such as sunflower, corn, soybean and paraffin oils, also varies among studies.

With n-3 PUFA supplementation alone, studies report no change in body weight (Table 2). One study in healthy adults supplemented with fish oil diets demonstrated decreased body fat mass, basal respiratory quotient and increased basal lipid oxidation when dietary intake was controlled [65]. Another study found reduction in fat mass along with significantly increased lean mass (fat-free mass) despite no alterations in total body mass, resting metabolic rate (RMR) or respiratory exchange ratio when compared to placebo supplementation [66]. Participant-reported diet diaries indicate significant reductions in carbohydrate, fat and total caloric intake with n-3 PUFA supplementation in one study [67], but others show no change in energy intake [68–71]. Since most studies only report total caloric intake, the effect of n-3 PUFA supplementation on macronutrient and energy intake should be repeated in larger studies to conclusively determine the role of n-3 PUFA in weight loss in humans.

5.1. Omega-3 PUFA in combination with dietary interventions

Weight loss results appear more promising when n-3 PUFA supplementation is combined with calorie restriction (Table 3), but it is difficult to draw conclusions due to the variety of calorie restriction programs in different studies. Greater improvements in

metabolic parameters, such as improved insulin resistance and decreased TGs, were attained with combined n-3 PUFA supplementation and calorie restriction compared to calorie restriction alone [72–74] or replacement of SFA [71]. Interestingly, results appear to be independent of the source, form or dose, i.e., different fish species (salmon, tuna, sardines, etc.) or fish oil capsules, of n-3 PUFA supplied [72,73,75]. This was confirmed by Thorsdottir et al., who compared the effects of various fish (cod or salmon) and fish oil (DHA/EPA capsules) in conjunction with 30% calorie restriction on weight loss in young, overweight adults for 8 weeks. After 4 weeks, men receiving cod, salmon or fish oil capsules lost approximately 1 kg more than those on 30% calorie restriction alone. The fish species and fish oil capsules supplied various amounts of n-3 PUFA: 0.3 g/day from cod, 3.0 g/day from salmon and 1.5 g/day from fish oil capsules, yet the n-3 PUFA dose did not influence weight loss outcomes. This suggests that variations in weight loss benefits may not depend solely on variations in n-3 PUFA dosages from study to study [76].

Rapid weight loss, induced by a very low calorie diet, alters adipose tissue and serum fatty acid composition [77,78]. Supplementation with n-3 PUFA during rapid weight loss increases serum n-3 PUFA concentrations [79] and may help prevent unfavorable changes in fatty acid tissue composition and essential fatty acid deficiency [77]. Some studies have utilized n-3 PUFA supplementation prior to a weight loss intervention, such as dietary restriction and/or an exercise regimen, and reported significant reductions in weight [80], while others observed no changes in body mass index (BMI) or body composition, particularly in insulin-resistant individuals [81]. Nonetheless, this type of study design should be refined and pursued further due to the relationship between tissue/plasma/erythrocyte n-3 PUFA concentrations and obesity. It will be important to verify if increasing n-3 PUFA concentrations prior to interventions would aid in weight loss and ameliorate obesity related metabolic dysfunctions.

Table 4
Effects of n-3 PUFA in addition to diet and/or exercise on body weight, body composition and energy intake in humans

N (M/F)	Participants Age (years)	n-3 PUFA (% in diet)	Control/diet intervention	Duration	Exercise	Body weight	Fat mass	Lean mass	Energy intake	Ref.
32 M	Healthy 19–34 years	4g n-3 PUFA	No supplementation	10 weeks	Aerobic 70%–85% HR max 1 h/3×/wk	NC	NC	NA	NC	[86]
45 F	Healthy 62–66 years	4 g n-3 PUFA 2 g n-3 0.4 g EPA 0.3 g DHA NA	No supplementation	10 weeks 12 weeks 12 weeks, 60 initial supplementation	Maintain Strength training 3×/wk Strength training 3×/wk	NC NC	NC NA	NA NA	NC NC	[215]
50 F	Healthy 65–70 years	Healthy diet		24 weeks	Resistance Training 2×/wk	NC	NA	+	NC	[88]
128 (40/88)	Overweight/obese, healthy 40–55 years	3 g EPA + DHA 5:1 (EPA:DHA)	Soybean and corn oil/ Nutrition counseling	24 weeks	150 min/week at 50%–85% of VO ₂ max	NC	NC	NA	NC	[83]
39 (6/33)	Mets 36–64 years	3 g FO 540 mg EPA 360 mg DHA	No supplementation/ Nutrition counseling	20 weeks	80 min 3×/wk walking 60 min resistance training 2×/wk	NC	NC	NA	NC	[84]
29 M	Obese, IR 32–65 years	1000 mg EPA 700 mg DHA	Glucose/starch	16 weeks, initial 4 week supplementation	3–5 walking sessions/wk at 50%–65% Hrmax	NC	NA	NA	NA	[81]
16 (6/11)	Overweight, hyperlipidemia, hypertensive 25–65 years	1.9 g n-3 PUFA 360 mg EPA 1.56 g DHA	Sunflower oil	12 weeks	Run/ walk 3×/wk for 45 min at 75% of APHRmax	NC	–	NC	NC	[87]
17 (5/11)	Overweight, hyperlipidemic, hypertensive 25–65 years	1.9 g n-3 PUFA 360 mg EPA 1.56 g DHA	Sunflower oil	12 weeks	none	NC	–	NC	NC	
7 (5/2)	Hyperlipidemic 27–63 years	50 ml FO 17% EPA 12% DHA	Corn oil	12 weeks	Walk/jog 3×/wk for 45–50 min at 70%–85% max HR	NC	–	NA	NC	[85]
7 (4/3)	Hyperlipidemic 27–63 years	50 ml FO 17% EPA 12% DHA	Corn oil		None	NC	NC	NA	NC	
20 F	Severely obese 37–60 years	2.8 g n-3 2:1 EPA: DHA	Placebo/ VLCD	3 weeks, inpatient	60 min/day light to moderate	–	NA	NA	NA	[82]
50 (27/23)	Healthy 65–77 years	3 g FO	Placebo	18 weeks	Lower-limb resistance training 2×/wk	NC	NA	NC	NA	[89]

APHRmax, age-predicted heart rate maximum calculated by $[208 - (0.7 \times \text{age})]$; HR, heart rate; VLCD, very-low calorie diet.

5.2. Omega-3 PUFA and exercise

Others have explored the influence of n-3 PUFA in conjunction with exercise and with or without a dietary intervention to determine if the addition of n-3 PUFA leads to greater weight loss (Table 4). With the addition of n-3 PUFA to an exercise regimen and dietary intervention, only one study has shown a decrease in body weight [82]. However, only a few such studies have been conducted, and the dietary intervention consisted of nutritional counseling rather than a prescribed diet [83,84].

The combined effects of n-3 PUFA and exercise are currently unknown. Well-designed placebo-controlled randomized clinical trials are lacking [85], as they require healthy and lean participants [86]. Differences in the intensity and forms of exercise (i.e., aerobic or resistance training) employed prevent valid comparisons across studies. The addition of n-3 PUFA to aerobic training without dietary intervention has resulted in decreases in fat mass [87]. Furthermore, the addition of n-3 PUFA to resistance training without dietary intervention resulted in increases in lean mass [88] and improved muscle quality [89].

5.3. Limitations in human studies

Overall, findings on the effects of n-3 PUFA in humans are inconclusive. Improvements in study design and analyses could help

resolve apparent inconsistencies in the effects of n-3 PUFA on weight and body composition. For example, sex, metabolic phenotype and geographic location should be taken into consideration in addition to n-3 PUFA supplementation. There is also a case for evaluating translation to real-world weight-loss diets, which are complicated by the need to control for eating behavior and physical activity. Even when participants are supplied with food, outpatient studies are difficult to translate because measurements of adherence to the recommended interventions [90] generally rely on self-reporting. Hence, studies using inpatient feeding and analysis of energy utilization should be carried out [91].

Failure to assess energy expenditure in human studies limits our understanding of the associations between n-3 PUFA status and decreased adiposity, weight loss and energy balance, especially when energy intake is unchanged. This highlights the need for tightly controlled studies, similar to that of Hall et al., to validate fuel partitioning and n-3 PUFA influence on metabolism in humans. Unfortunately, work of this nature is expensive, labor intensive and generally of short duration with small sample sizes [91].

Other limitations on current human studies of n-3 PUFA supplementation include the use of less reliable anthropometric methods [92] and failure to dose according to body weight to meet the threshold of tissue membrane n-3 PUFA phospholipid enrichment [93]. Finally, it is of utmost importance to utilize a standardized method, such as the omega-3 index, to assess n-3 PUFA status, the

biological effects of n-3 PUFA and n-3 PUFA related metabolites [34]. Future human studies should employ this method to verify that n-3 PUFA consumption parallels n-3 PUFA concentrations in the body.

6. Mechanisms by which n-3 PUFA improve adiposity and metabolic disorders

There are several proposed mechanisms by which n-3 PUFA could work in reducing body weight and improving the metabolic profile (Fig. 3). These include alterations in adipose tissue gene expression; changes in adipokine release; adipokine-mediated or adipokine-related pathways; appetite suppression; alterations in carbohydrate metabolism; increases in fat oxidation; increases in energy expenditure (possibly through thermogenesis); activating mechanisms involved in muscle anabolism; and, lastly, influence on epigenetics.

6.1. Omega-3 PUFA and adipogenesis

Adipose tissue expansion in obesity occurs via adipocyte hypertrophy (enlargement of adipocytes) and hyperplasia (increase in adipocyte number due to adipogenesis). The latter is associated with smaller adipocyte size and a metabolically healthy phenotype. Both n-3 and n-6 PUFAs can bind and/or regulate transcriptional factors that control genes involved in preadipocyte differentiation. PUFAs, particularly AA and its metabolites, serve as ligands for peroxisome proliferator-activated receptors (PPAR) gamma (PPAR γ) and delta (PPAR δ) to induce fat cell differentiation and accelerate maturation by elevating lipoprotein lipase expression *in vitro* [94,95]. Elevated concentrations of n-6 and n-3 PUFA in human subcutaneous tissue correlate with reduced adipocyte size; increased SFA concentrations lead to increased fat cell size [96]. Differences in fatty acid concentrations are more strongly associated with abdominal subcutaneous than visceral adipose tissue [35].

Studies performed in clonal adipocytes (3T3-L1) also demonstrate up-regulation in PPAR γ expression, adipogenesis and lipid droplet formation after the addition of n-3 PUFA [43,97]. Taken together, these studies suggest that n-3 PUFAs promote adipogenesis and a healthy expansion of adipose tissue during positive energy balance, promoting a metabolically healthy phenotype.

6.2. Adipose tissue inflammation

Chronic low-grade inflammation and changes in adipokine patterns are key factors in the pathogenesis of metabolic derangements in obesity (Fig. 1). Indeed, a relationship exists between BMI, body fat percentage and inflammatory markers [98]. Omega-3 PUFAs inhibit nuclear transcription factor kappa B, a key transcription factor in cytokine gene expression and inflammation [99]. In humans and *in vitro*, n-3 PUFAs also have a documented role in reducing cytokines, including IL-1 [100,101], IL-6 [102] and TNF- α [100,103], which are all elevated in obesity. (For an extensive review of mechanisms of n-3 PUFA and adipose tissue inflammation, see Kalupahana et al., 2011).

Omega-3 PUFAs act as agonists for different members of the free fatty acid receptor family (FFARs) present on a variety of cell types involved in both energy homeostasis and the inflammatory response. A number of saturated and unsaturated long-chain fatty acids can activate FFAR1 and FFAR4 [104,105]. Agonist stimulation that impedes the inflammatory response occurs through activation of the G-protein-independent signaling pathway through interaction with β -arrestin proteins, which may further interact with the transforming growth factor kinase protein (TAK1) and binding protein (TAB-1). Stimulation of FFAR4 or β -arrestin inhibits lipopolysaccharide (LPS)-mediated release of inflammatory cytokines, including TNF- α and IL-6 in the macrophage-like cell line RAW264.7. In fact, decreased macrophage infiltration into adipose tissue has been shown in mice fed an n-3-PUFA-enriched diet, possibly via activation of FFAR4 (G-protein-coupled receptor 120). Since n-3 PUFAs are unable to reduce

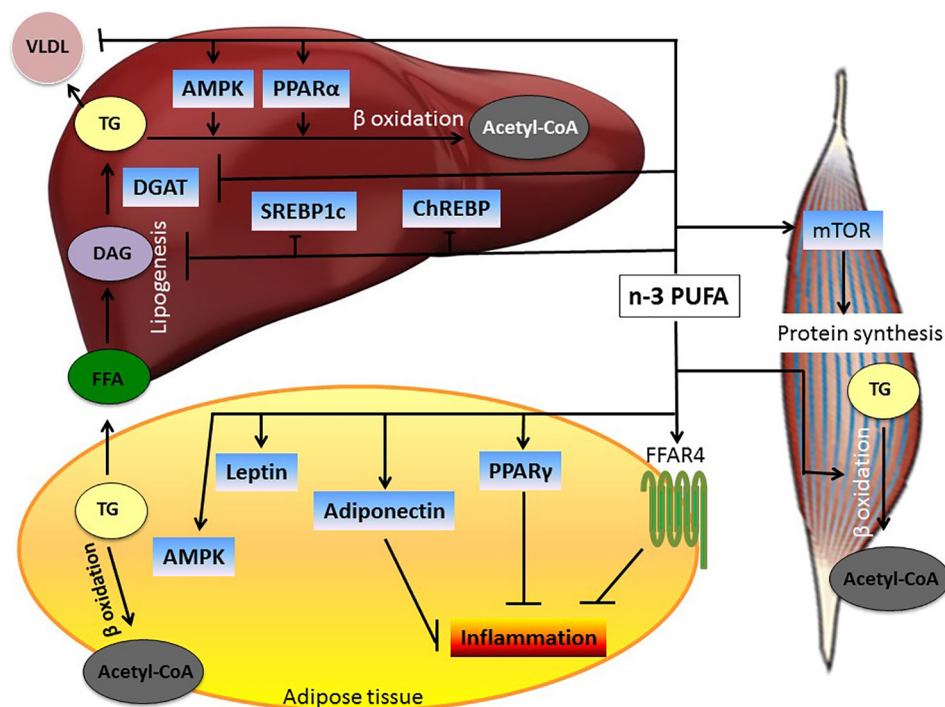


Fig. 3. Mechanisms mediating effects of n-3 PUFA on liver, adipose tissue and skeletal muscle metabolism. Omega-3 PUFAs increase fatty acid oxidation in the liver, adipose tissue and skeletal muscle, thus limiting fat storage in these tissues. Omega-3 PUFAs also decrease the production and release of proinflammatory adipokines. In skeletal muscle, n-3 PUFAs promote protein synthesis. All mechanisms depicted here contribute to an improved metabolic profile.

adipose tissue macrophage infiltration in FFAR4 knockout mice, this highlights the mechanistic importance of FFAR4 in mediating the anti-inflammatory effects of n-3 PUFA [106]. Furthermore, fish oil supplementation (4 g n-3 PUFA/day) in obese humans has been associated with decreased M1 macrophage presence in adipose tissue and subsequent decreases in proinflammatory markers, such as IL-8 [107]. Accordingly, monocytes differentiate preferentially into M1 macrophages when treated with human postprandial triglyceride-rich lipoproteins following a meal rich in saturated fatty acids, versus a meal high in MUFA or PUFA, after which they shift towards M2 macrophages [108].

Furthermore, n-3 PUFAs halt inflammatory processes by inhibiting activation of the NLRP3 (nucleotide-binding oligomerization domain-like receptor; NLR family, pyrin domain containing 3) inflammasome via an arrestin-FFAR4-dependent pathway [109], which triggers a caspase-dependent cascade, resulting in the release of proinflammatory cytokines [110]. The n-3 PUFA DHA acts through FFAR1 or FFAR4 to suppress caspase-1 activity via formation of a β -arrestin-2/NLRP3 or NLRP1b complex and thus decrease the release of proinflammatory cytokines [109].

Omega-3 PUFAs also influence lipid rafts, which are cholesterol- and sphingolipid-rich areas of the plasma membrane [111] that can form signaling platforms [112,113]. Incorporation of n-3 PUFAs into plasma membranes disrupts lipid rafts [114] and hence could mediate anti-inflammatory and antichemotactic n-3 PUFA properties.

6.3. Adipokine secretion

Several studies have shown that n-3 PUFAs modulate adipokine secretion. Obese individuals have high plasma leptin levels [115] suggestive of leptin resistance. Conversely, weight loss leads to parallel decreases in plasma leptin levels [116]. This weight-loss-associated decrease in leptin could contribute to hunger and a lower metabolic rate and ultimately lead to weight regain [117]. EPA supplementation attenuates the decrease in blood leptin levels that occurs during weight loss in obese women, suggesting a potentially significant role of EPA in weight loss maintenance [118]. Indeed, EPA increases the production of leptin in rodents and cultured adipocytes [37,119], suggesting a direct effect of n-3 PUFA on leptin production. However, the few studies that have assessed the role of n-3 PUFA in weight maintenance have found no significant effect on weight or blood leptin concentrations between n-3-PUFA-supplemented subjects compared to other weight loss groups [120,121]. Omega-3-PUFA-mediated effects on leptin are dependent on a number of factors, such as diet composition and energy balance, which could cause conflicting results.

Independent of body weight, both animal [37,48,122] and human [123,124] studies have found significant increases in blood levels of the insulin-sensitizing adipokine, adiponectin, following n-3 PUFA consumption. EPA appears to regulate adiponectin levels at the translational or posttranslational level rather than at the transcriptional level [123]. It has been proposed that the anti-inflammatory properties of n-3 PUFA supplementation induce an increase in adipocyte adiponectin production [123] and improve leptin sensitivity [125]. This type of interplay could have a significant influence on body weight regulation. An inverse relationship between serum adiponectin concentrations and TNF- α has also been demonstrated in ob/ob mice [123] and in overweight and insulin-resistant children following n-3 PUFA supplementation [126].

Fatty acid-binding proteins are cytosolic proteins that bind long-chain fatty acids and promote transport to several organelles. Fatty acid-binding protein 4 (FABP4; adipocyte FABP, A-FABP; or aP2) is secreted from both macrophages and adipocytes and functions as an adipokine [127]. An elevated FABP4 serum concentration is associated with obesity, insulin resistance and hypertension [128]. Adipocytes

are the predominant contributors of circulating FABP4. During lipolysis, FABP4 functions in a nonclassical secretion pathway [129]. Omega-3 PUFA dose-dependently reduced FABP4 secretion in 3T3-L1 adipocytes and reduced serum FABP4 concentrations in humans [130]. Omega-3-PUFA-mediated reductions in FABP4 could also be due in part to reduced expression of transcription factors involved in adipocyte differentiation, including PPAR γ 2 and C/EBP α [130]. Another possible mechanism by which FABP4 levels are lowered by n-3 PUFA is through the β -adrenergic receptor [129] since n-3 PUFAs reduce sympathetic nerve activity and thus may lower FABP4 serum level [130]. Taken together, n-3 PUFAs modulate adipokine secretion by exerting anti-inflammatory effects and promoting a metabolically healthy phenotype.

6.4. Appetite suppression

In addition to leptin, central and peripheral peptides and hormones involved in food intake and energy expenditure signaling pathways are targets for n-3-PUFA-derived endocannabinoids and thus may be implicated in the prevention and treatment of obesity. A subanalysis of the study conducted by Thorsdottir et al. reported elevated sensations of fullness in the participants who consumed higher n-3 PUFA content meals (fatty fish and fish oil) compared to those who consumed lower n-3 PUFA content meals (control and lean fish) both immediately and 2 h after consuming the meal. Feelings of hunger were consistently lower in participants who ate the meal higher in n-3 PUFA content [131]. Therefore, it is possible that increased feelings of satiety following a meal high in n-3 PUFA content could aid weight loss by reducing subsequent food intake. Appetite suppression could also be mediated through FFAR4 (GPR 120). Omega-3 PUFAs are agonists for FFAR4 [132], which elicits the secretion of cholecystokinin, a peptide hormone that is synthesized and released from the small intestine and has roles in hunger suppression [133].

6.5. Insulin resistance

Adipose tissue inflammation is at least in part responsible for obesity-associated insulin resistance. Since n-3 PUFAs alleviate adipose tissue inflammation as outlined above, reducing adipose tissue inflammation is a possible mechanism for n-3-PUFA-associated improvements in insulin sensitivity observed in animal models.

Hepatic insulin resistance in which both glucose production and lipogenesis are increased is characteristic of the metabolic dysregulation seen in obesity and T2DM. This dysregulation is attributed to reductions in proximal insulin signaling kinases, such as P13K and AKT, which hinder gluconeogenesis, as well as activation of mTORC1 and p70S6K, which control lipogenesis [134,135].

Fibroblast growth factor (FGF) 21, which is produced by the liver, adipose tissue and skeletal muscle, has been shown to reduce both hepatic glucose production and plasma glucose levels, while it also increases insulin sensitivity and adipocyte glucose uptake [136]. Circulating FGF21 levels are elevated in diet-induced obese mice [137] and obese and type 2 diabetic humans [138], suggesting obesity-related FGF21 resistance. Omega-3 PUFAs attenuate HF-diet-induced increases in FGF21 [139] with associated reductions in hyperglycemia, hypertriglyceridemia and plasma insulin levels [140,141]. This could be a potential mechanism by which n-3 PUFAs improve insulin resistance.

Omega-3 PUFA supplementation prevents insulin resistance in muscle of rats fed an HF diet [142], partly by improving glycogen synthesis [143]. Omega-3 PUFAs also decrease fat content in muscle and maintain normal PI3K activity and expression and transcription of GLUT 4 receptors in muscle and thus improve myotubule glucose uptake. Omega-3 PUFAs also promote inhibition of hepatic glucose production [142].

Hence, n-3 PUFAs may be a valuable nutritional tool for preventing or diminishing muscular and hepatic insulin resistance associated with obesity. However, n-3 PUFAs appear ineffective once T2DM is established [144].

6.6. Lipid metabolism

In both animal and human studies of n-3 PUFA supplementation, reductions in weight or fat mass were not accompanied by changes in energy intake (Tables 1–4). Omega-3 PUFAs can partition dietary fuel away from storage and toward oxidation by suppressing lipogenic genes and activating genes that encode for mitochondrial and peroxisomal fatty acid oxidation in both the liver and muscle.

Given their cardioprotective properties, n-3 PUFAs can improve endothelial function in patients with varying metabolic profiles [145], possibly through increased production of nitric oxide [146]. Furthermore, during exercise, fish oil has been shown to increase arterial dilation and blood flow to skeletal muscle [147]. Hence, improved blood flow may increase the delivery of fats to be utilized as energy in skeletal muscle, especially during exercise.

Regulation of lipid metabolism may vary by n-3 PUFA type, as well as by fat depot. For example, EPA is preferentially directed towards β -oxidation, while DHA and DPA are spared from catabolism and deposited in tissues [148]. Moreover, gene expression of fatty acid synthase [149], hormone-sensitive lipase, lipoprotein lipase and phosphoenolpyruvate carboxykinase in retroperitoneal fat is decreased with DHA and mixed EPA/DHA supplementation but not with EPA supplementation alone [41].

Portions of hepatic TG are secreted via very low density lipoprotein (VLDL), which delivers TG to peripheral tissues, such as WAT. Hepatic VLDL secretion is enhanced in obese individuals [150] possibly due to increased fatty acid delivery, elevated glucose and insulin concentrations, as well as impaired fat oxidation, which increases fatty acid esterification into TG [151]. Omega-3 PUFAs reduce lipogenesis and reduce hepatic VLDL secretion [51]. *In vitro*, n-3-PUFA-treated HepG2 cells have decreased hepatic VLDL secretion [152] and reduced apolipoprotein B100 production [153]. This has been validated in both DHA- and n-3-PUFA-supplemented animals [154]. Hence, through inhibition of VLDL formation, n-3 PUFAs could limit the supply of fatty acids to adipocytes and thereby limit adipocyte size and mass. In a deregulated system, n-3 PUFAs would also limit the amount of fatty acids delivered to muscle and liver. Additionally, in animal models, n-3 PUFAs modulate cholesteryl ester transfer protein mediated exchanges, resulting in increased blood HDL cholesterol and possibly apolipoprotein A-1 concentrations [155,156].

Omega-3 PUFAs alter expression and nuclear localization of both the transcription factor sterol-regulatory element-binding protein-1 (SREBP-1) and the carbohydrate response element binding protein (ChREBP), which control several lipogenic genes, including those regulating cholesterol and fatty acid synthesis [154,157]. Nuclear translocation of ChREBP is inhibited by n-3 PUFAs and thus results in reduced expression of lipogenic and glycolytic genes, including FAS and pyruvate kinase respectively [158]. Furthermore, n-3 PUFAs suppress hepatic lipogenesis by reducing both messenger RNA (mRNA) and active protein expression of SREBP-1c, which results in reduced expression of many genes involved in lipogenesis, including FAS and acetyl-coA carboxylase [159–161]. Reduced SREBP-1c expression, via n-3 PUFA, has been attributed to inhibited transcription of nascent precursor SREBP-1c, accelerated transcript decay and reduced levels of the mature cleaved form of SREBP-1c [159,160], possibly through inhibition of proteolytic processing and reduced feed-forward activation of the *Srebf1* gene. This inhibition could be due to interference with insulin signaling pathways, which promotes the proteolytic processing of SREBP-1c, potentially via an AKT-dependent mechanism [162,163].

The role of liver X receptor (LXR) is controversial. *In vivo*, EPA suppression of SREBP-1c promoter activity is dependent upon an intact SRE but not LXR response elements, suggesting that decreased transcription of nascent SREBP-1c with n-3 PUFA treatment results from decreased availability and thus reduced feed-forward activation [163]. In contrast, others indicate a role in the inhibition of LXR α in reduced SREBP-1c expression with n-3 PUFA, but this may be dependent upon cell types [164]. Accelerated degradation of SREBP-1c mRNA has also been proposed as a mechanism for reduced SREBP-1c expression [165]. Omega-3 PUFAs inhibit SREBP-1c cleavage processing, but the cleavage sites are unknown [166].

Activation of AMP-activated protein kinase by n-3 PUFA can also suppress SREBP-1c cleavage and nuclear translocation, perhaps via serine phosphorylation and/or by blocking activation of the insulin-responsive mechanistic target of rapamycin complex 1 (mTORC1)/S6K-signaling pathway [167]. SREBP-1c synthesis, transport and maturation are increased with insulin [162].

PUFAs, prostaglandins and leukotrienes can all act as ligands for PPARs. PPARs are transcription factors that form heterodimers with retinoid X receptors in the promoter regions of several genes involved in lipid and glucose metabolism [168,169]. For example, n-3 PUFA activation of PPAR α decreases lipogenesis by suppressing FAS activity [161,170]. However, lipogenesis suppression by n-3 PUFA does not require PPAR α activation [171].

PPAR γ acts as a master regulator of adipogenesis and controls several genes and adipokines in lipid and glucose metabolism. Omega-3 PUFAs act as ligands for PPAR γ and modulate several PPAR γ target genes in mice [172] and 3T3-L1 adipocytes [97]. Omega-3 PUFAs enhance PPAR γ binding to PPAR-response element in the promoter region of vascular endothelial growth factor-A, which promotes adipogenesis and alleviates hypoxia-induced adipocyte inflammation and insulin resistance [173]. It has been suggested that PPAR γ plays a significant role in the ability of n-3 PUFA, specifically DHA, to stimulate M2 macrophage polarization and thereby reduce inflammation since these results are not seen in PPAR γ knockdown RAW264.7 cells [174].

Omega-3 PUFAs have been shown to increase mitochondrial biogenesis and fatty acid oxidation in the liver [175,176], adipose tissue [43] and small intestine [177] of rodents, possibly through PPAR α and Cox3 induction [175,178,179]. PUFA-controlled genes involved in lipid oxidation and thermogenesis include mitochondrial HMG-CoA synthase [180], peroxisomal acyl-CoA oxidase [64,181], hepatic CPT-1 [154], FABP [127] and fatty acid transporter proteins [182].

Activation of PPAR α can also increase fatty acid oxidation. Increases in fatty acid oxidation by n-3 PUFA may also be mediated by AMPK, a known regulator of cellular energy metabolism. AMPK up-regulation by n-3 PUFA has been demonstrated in both adipose tissue and cultured adipocytes [55].

Taken together, n-3 PUFAs regulate lipid metabolism, favoring fatty acid oxidation and suppression of lipogenesis and leading to a favorable lipid profile and adipocyte metabolism.

6.7. Thermogenesis

Many have examined cold- and diet-induced thermogenesis mediated by mitochondrial uncoupling proteins (UCPs) in the presence of n-3 PUFA [43,48,54]. UCPs are inner mitochondrial proteins that function to transport hydrogen ions across the mitochondrial inner membrane. We have recently shown that BAT from EPA-supplemented mice expresses higher levels of thermogenic genes, such as PRDM16, peroxisome proliferator-activated receptor-gamma coactivator-1alpha and UCP1 [183].

Omega-3 PUFAs increase mitochondrial oxidative capacity in WAT [43] and skeletal muscle, possibly through UCP-3 up-regulation [48], but not in BAT or liver [43]. However, because most studies were carried out at 20°C, it is unclear whether increases in mitochondrial oxidative capacity

are n-3 PUFA mediated or cold induced. Janovska et al. reported no differences in body weight but decreased epididymal fat mass after feeding an HF diet supplemented with n-3 PUFA in mice kept at 30°C, indicating that n-3 PUFA could attenuate body fat accumulation even at thermoneutrality and independent of cold-induced thermogenesis [52]. Mechanisms underlying the role of n-3 PUFA in possible induction of energy expenditure and prevention of body fat accumulation should be investigated further at various temperatures since thermogenic markers are activated even at 22°C [183].

6.8. Lean mass

The mechanism by which n-3 PUFAs have the potential to increase lean mass is not fully understood but likely involves both catabolic and anabolic pathways. Increased lean mass would result in improved body composition and possibly improved metabolism. Even though increases in RMR have been demonstrated with increases in lean mass [184], post-n-3 PUFA supplementation increases in lean mass are not always accompanied by increases in RMR [66]. Future studies are needed to examine the relationship between n-3 PUFA changes in lean mass in relation to RMR since metabolic rate significantly influences body weight.

Omega-3 PUFAs have been shown to attenuate muscle protein breakdown in isolated muscles of mice [185]. Increases in protein synthesis may be mediated by n-3 PUFA activation of the mTOR-p70s6k signaling pathway [186], a key pathway in muscle cell growth. Similarly, Clark et al. reported increased whole-body protein turnover under insulin stimulation but did not see significant increases in lean mass following 9 months of n-3 PUFA supplementation [187]. Certainly, changes in protein dynamics may not translate to increases in protein mass.

One possible mechanism of n-3 PUFA in increasing lean mass is the alteration of protein dynamics related to n-3 PUFA anti-inflammatory properties [66] since proinflammatory cytokines like TNF- α can increase protein degradation via ATP-ubiquitin-dependent pathways [188].

Another such potential mechanism relates to the ability of n-3 PUFA to lower cortisol levels [189]. Noreen et al. reported decreases in cortisol with n-3 PUFA supplementation but noted a significant correlation between cortisol level and changes in body composition [66]. Others have shown that a reduction in fat mass does not lower cortisol production [190]. Hence, n-3 PUFA supplementation may modulate cortisol levels so as to improve body composition [66]. Furthermore, proinflammatory cytokines such as IL-6 have been shown to increase blood levels of cortisol [191], which increases protein catabolism [192]. Hence, anti-inflammatory properties of n-3 PUFA could aid in disrupting this pathway. However, increased muscle protein synthesis with n-3 PUFA supplementation is not likely mediated by changes in inflammation in a healthy population [186]. Further investigations are needed to elucidate the mechanisms by which n-3 PUFAs alter protein dynamics to increase lean mass.

6.9. Epigenetics and microRNA

Epigenetics may be an important contributor to many chronic diseases, including obesity [193,194]. Limited studies have examined n-3 PUFA and epigenetic modifications even though the expression of several genes involved in metabolic homeostasis is regulated by DNA methylation. The few that have been conducted report conflicting evidence for DNA methylation and n-3 PUFA. In a population-based study, n-3 PUFA intake was associated with DNA methylation in Alaska Yup'ik people [195]. A few studies have reported that fish oil supplementation did not alter the methylation pattern of genes [196,197], including leptin and the leptin receptor, in mouse epididymal fat [196]. It may be that n-3 PUFAs work through epigenetic mechanisms other than methylation [197]. In diet-

induced obese mice, leptin expression may be regulated by n-3 PUFA via changes in methyl-CpG-binding domain protein 2 and histone modifications [198]. Since an HF diet has been shown to cause changes in the methylation of gene-specific promoter regions in the liver [199] and WAT [200], the influence of n-3 PUFAs on epigenetic modifications warrants further investigation.

MicroRNAs (miRNAs) are short noncoding RNAs that act as posttranscriptional regulators of genes by acting as sequence-specific inhibitors of mRNA. These miRNAs target transcription factors to indirectly affect entire signaling pathways. It has been documented that miRNAs act as key regulators in the pathogenesis of metabolic disease by affecting inflammation [201] and lipid metabolism [202].

Recent studies have shown n-3 PUFA to modulate miRNA expression [201,203,204]. A diet enriched in PUFA correlated to changes in circulating miRNAs, specifically miR-106a, along with changes in other miRNAs related to lipid metabolism and adipokine secretion in healthy women [203]. In animal models, n-3 PUFAs suppress inflammation through down-regulation of miR-19b-3p, -146b-5p and -183-5p by targeting toll-like receptor, NOD-like receptor, RIG-I-like receptor, mitogen-activated protein kinase and transforming growth factor- β pathways [201]. In obese rats, DHA has been shown to counteract obesity-related increases in hepatic miR-33a and miR-122, thus improving lipid metabolism via decreased SREBP2 and FAS expression, respectively [204]. Therefore, fully characterizing n-3 PUFA modulation of miRNA involved in key pathways, such as lipid metabolism and inflammation, is warranted and could play a key role in targeting MetS and obesity-related therapies.

7. Future perspectives

Both animal and human studies have examined the beneficial effects of combining n-3 PUFA with other dietary supplements and pharmaceuticals including antidiabetic drugs, L-alanyl-L-glutamine [205], as well as α -lipoic acid [118] and krill oil [206]. Animal studies using the combination of n-3 PUFA and rosiglitazone have reported significantly greater reductions in body weight [63,143], enhanced oxidation of fatty acids [207] and counteraction of lipogenesis than with rosiglitazone therapy alone [63]. Omega-3 PUFA supplementation in addition to antidiabetic pharmaceuticals could attenuate body weight gain caused by pharmaceuticals and should be further investigated [208].

8. Conclusions

The management of obesity has shifted from a narrow focus on BMI to the wider field that includes the complications of obesity, with the goal to reduce obesity-associated comorbidities [209]. While n-3 PUFAs have not yet shown consistent benefits in terms of weight loss in humans, improvements in the metabolic profile of obese individuals have been demonstrated. Therefore, n-3 PUFAs may be an important adjunct to obesity management along with lifestyle modification and pharmacotherapy. Further study of the genetic and epigenetic molecular targets related to metabolism, appetite and energetics could aid the discovery of novel therapeutic targets for obesity-associated metabolic disorders.

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