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Adaptive thermogenesis by dietary n-3 polyunsaturated fatty acids: emerging evidence and mechanisms

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Abstract

Brown/beige fat plays a crucial role in maintaining energy homeostasis through non-shivering thermogenesis in response to cold temperature and excess nutrition (adaptive thermogenesis). Although numerous molecular and genetic regulators have been identified, relatively little information is available regarding thermogenic dietary molecules. Recently, a growing body of evidence suggests that high consumption of n-3 polyunsaturated fatty acids (PUFA) or activation of GPR120, a membrane receptor of n-3 PUFA, stimulate adaptive thermogenesis. In this review, we summarize the emerging evidence that n-3 PUFA promote brown/beige fat formation and highlight the potential mechanisms whereby n-3 PUFA require GPR120 as a signaling platform or act independently. Human clinical trials are revisited in the context of energy expenditure. Additionally, we explore some future perspective that n-3 PUFA intake might be a useful strategy to boost or sustain metabolic activities of brown/beige fat at different lifecycle stages of pregnancy and senescence. Given that a high ratio of n-6/n-3 PUFA intake is associated with the development of obesity and type 2 diabetes, understanding the impact of n-6/n-3 ratio on energy expenditure and adaptive thermogenesis will inform the implementation of a novel nutritional strategy for preventing obesity.

Keywords

Fish oil; GPR120; n-6/n-3 ratio; adaptive thermogenesis; brown adipocyte; beige adipocytes; thermogenic diet; UCP1

1. Introduction

Adaptive thermogenesis is an energy-demanding process in which futile uncoupled-respiration releases the mitochondrial proton gradient as heat. It is regulated by a range of intrinsic and extrinsic factors including daily diet [1]. Diet plays a first-line role in regulating

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energy balance and adiposity. Beyond the obvious strategy of calorie restriction, emerging evidence supports the thermogenic function of several food-derived components. Dietary intervention is more likely a safe approach to modulate brown/beige energetics compared to targeted activation of the β3-adrenergic receptor (ADRB3) by pharmacological interventions due to the increased risk of cardiovascular side effects [2]. Nearly a dozen dietary polyphenolic compounds have been identified as candidate molecules to stimulate brown/beige activation [1, 3]. For example, capsaicin [4], green tea catechins [5–7], resveratrol [8, 9], quercetin [10, 11], flavan-3-ols [12], and berberine [13] are reported to possess brown stimulatory property. However, low bioavailability of dietary polyphenols poses a challenge in launching a readily translatable anti-obesity approach through diet-induced thermogenesis.

One promising dietary factor to promote adaptive thermogenesis is fish oil. The metabolic benefits of fish oil were first identified in the early 1970's in the Greenlandic Inuit who have adapted to the extremely cold temperature in the Arctic region. Danish researchers reported that the cardio-protective effects of the Greenlandic Inuit diet are attributed to the specialized fish diet enriched with n-3 polyunsaturated fatty acids (PUFA), in particular eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) [14]. Fish oil supplementation provides various health benefits in a tissue-specific manner; n-3 PUFA have been shown to increase energy expenditure in muscle [15], decrease inflammation in immune cells [16], promote insulin secretion in pancreatic beta cells [17], and attenuate pro-atherogenic lipoprotein production in the liver [18]. The metabolic benefits derived from fish oil resemble the adaptive metabolic responses upon brown/beige fat activation. This implies that the previously reported metabolic benefits of fish oil may be, at least partly, through adaptive thermogenesis. Very recently, n-3 PUFA intake has gained attention as a dietary regimen to promote thermogenesis. A series of studies published by different groups has revealed the novel functions of fish oil in activating brown fat as well as recruiting beige fat within WAT [19–21].

The prime goal of this review is to compile recent evidence that contributes to our understanding of the thermogenic function of dietary n-3 PUFA. We also define the potential mechanisms underlying the thermogenic function of n-3 PUFA. Given that G-protein coupled receptor 120 (GPR120), also known as free fatty acid receptor 4 (FFAR4), is a well-known membrane receptor for n-3 PUFA [22], we highlight the mechanism whereby GPR120 serves as a signaling platform to regulate transcriptional, immunological, and endocrine function for thermogenic activation of brown/beige fat. We also delineate the GPR120-independent mechanisms by which n-3 PUFA intake stimulates sensory neurons in the gut, activating the sympathetic nervous system (SNS), and n-6/n-3 ratio modulates the thermogenic potential of adipocytes through different eicosanoid formation. Next, we revisit the human clinical trials with fish oil in the context of weight loss and energy expenditure to gain general insights into the thermogenic function of n-3 PUFA in humans. In the last part of this review, we briefly provide physiological perspectives that n-3 PUFA supplementation may improve metabolic activity at different lifecycle states. We discuss the likelihood that maternal n-3 PUFA intake reinforces programming of fetal brown fat development and rejuvenates beige fat thermogenesis in the elderly, helping to attenuate age-related pathology.

2. Proposed mechanism established from animal studies

2.1. Thermogenic function of fish oil through GPR120 activation

G-protein-coupled receptor 120 (GPR120) is a functional receptor/sensor of n-3 fatty acids (FA) [22]. Upon binding of n-3 PUFA, GPR120 activates the heterodimeric Gαq subunit and subsequently induces diverse cellular responses via several second messengers such as $[Ca^{2+}]_i$, cAMP, and diacylglycerol (DAG) (reviewed in [22, 23]). Adipocytes, but not preadipocytes, express high levels of GPR120 in the rank order of BAT≫ iWAT ~ eWAT > mWAT [21]. In this section, we focus on the role of GPR120 activation by fish oil in governing brown/beige fat activation.

A. Activation of brown fat-specific transcriptional program by n-3 PUFA— Humans contain a measurable amount of classical brown fat that expresses constitutively active uncoupling protein 1 (UCP1) compared to beige fat that has temporal and reversible UCP1 activation dependent on environmental stimuli such as cold temperature and excess nutrition [24]. Provided the continuous nature of the thermogenic function, a small increase of BAT mass and/or activity are thought to exert a profound impact on overall energy expenditure. Classical brown fat is found in certain anatomical depots such as the cervical, supraclavicular, perirenal, axillary, and paravertebral areas [25].

Several research groups including our laboratory have tested the possibility that dietary fish oil or GPR120 agonists augment the mass and/or activity of classical brown fat [19, 21, 26, 27]. As an *in vitro* model of brown adipocytes, Kim et al. isolated brown fat precursor cells from the interscapular BAT (iBAT), and then n-3 PUFA (*i.e.,* EPA and DHA) were added during differentiation and compared with other fatty acids (oleic acid and palmitic acid). Treatment with 100 μM of EPA and DHA, but not oleic and palmitic acid, significantly promoted brown fat-specific transcriptional programming, resulting in an increase of uncoupled respiration [19]. Supporting these results, Pahlavani et al. showed that EPA (50–100 μM) treatment increased thermogenesis in HIB1B cells, brown preadipocytes derived from a murine brown fat tumor [27]. In addition, Kim el al. demonstrated that EPA-induced transcriptional activation of brown adipogenesis relied on microRNA (miRNA)-mediated epigenetic mechanisms in primary murine brown adipocytes. Functional clusters of brown fat-specific miRNAs including miR193b/365, miR30b, and miR378 were induced by EPA treatment [19]. More importantly, transcriptional activation of brown adipogenesis as well as miRNA production were dependent on GPR120 signaling. Stimulation with GW9508, a GPR120 agonist, recapitulated the effects of EPA. Conversely, depleting GPR120 expression by siRNA [19] or genetic deletion [21], attenuated EPA-mediated activation of brown adipogenesis. These data suggest that GPR120 signaling precedes the transcriptional activation of brown adipogenesis, in part through miRNAs-mediated epigenetic mechanisms.

The effects of EPA on BAT thermogenesis were validated in HF diet-fed C57BL/6 mice. EPA supplementation, either provided as pure EPA [27, 28] or fish oil formulation [19–21, 26], reduced HF-diet induced obesity and metabolic dysfunction by increasing thermogenic energy expenditure. In addition, these in vivo metabolic benefits were concurrent with GPR120 activation and brown-specific miRNA production [19]. A further metabolic insight into the role of GPR120 on BAT thermogenesis was found in GPR120 null mice. These mice

failed to upregulate cold-induced BAT genes and demonstrated loss of brown fat-specific gene profiles and morphology, and ineffective maintenance of body temperature with cold exposure [29]. Taken together, these results suggest a novel epigenetic signaling axis of EPA/GPR120/miRNAs is involved in regulating brown fat function [19]. In addition, GPR120-dependent FGF21(fibroblast growth factor 21) secretion was proposed to propagate the BAT-driven thermogenic responses to other tissues, e.g., liver and muscle, via autocrine and paracrine signaling of FGF21 [21].

Rosell et al. report the very intriguing intrinsic feature of GPR120 in BAT [30]. Cold treatment itself promotes ~2-fold induction of GPR120 in BAT, but not in WAT [30], suggesting that the intrinsic ligand for GPR120 may be synthesized in brown fat as a part of cold adaptation. From this aspect, it is worthwhile to dissect BAT lipid metabolism during cold adaptation. In fact, n-3 PUFA is endogenously synthesized in brown fat, but not in white fat, in mice and rats [31, 32]. In addition, Inuits, indigenous people of the Canadian Arctic, possess selected alleles of FA desaturases, thereby facilitating endogenous n-3 PUFA synthesis [33]. Moreover, an induction of GPR120 by fish oil intake synergistically promotes thermogenesis compared to cold exposure alone [19, 30]. The elevated levels of n-3 FA in BAT via endogenous biosynthesis as well as high fish consumption would synergistically contribute to keeping Inuits warm against cold temperature by promoting brown thermogenesis. Further research is warranted to understand the exact nature of cold-induced GPR120 induction in BAT in the context of lipid profile changes, n-6/n-3 ratio, membrane fluidity, and thermogenic heat release.

In conclusion, GPR120 activation, either by endogenous synthesis of n-3 PUFA in cold or by dietary n-3 supplementation, plays an essential role in boosting brown fat-specific transcriptional programming including miRNA-mediated epigenetic mechanisms and FGF21 signaling (Figure 1A).

B. Role of GPR120 activation in beige fat differentiation by n-3 PUFA—Oh et al. first demonstrated that white adipocytes express high levels of GPR120, but not GPR40 [34]. The stimulation of GPR120 by n-3 PUFA, or its chemical agonist, promotes glucose uptake by sensitizing insulin signaling [22]. Supporting this notion, genetic ablation of GPR120 in mice results in hepatic steatosis and insulin resistance [35]. In humans, lack of GPR120 signaling activity due to a genetic mutation in the GPR120 gene (p.R2700H) is correlated with increased risk of obesity [35]. Despite several controversies surrounding the anti-obesogenic role of GPR120 in humans, evidence derived from experimental animals indicates that GPR120 activation increases lipid combustion and reduces adiposity.

Several groups of scientists provided direct evidence that fish oil promotes beige fat development from adipogenic precursor cells [20, 29, 36–38]. Zhao et al. first reported that 200 μM EPA treatment of stromal vascular (SV) cells isolated from mouse inguinal fat effectively induced beige-specific signature gene profiles, and increased mitochondrial energy expenditure [37]. Laigelesia et al. investigated the metabolic effects of EPA (100–200μM) on human adipogenic stem cells isolated from subcutaneous fat. Incubation with EPA (100–200μM) during adipogenic differentiation promoted mitochondrial biogenesis and induced thermogenic gene expression and specific beige markers such as *Cd137* [29].

Similarly, Fleckenstein-Elsen et al. demonstrated that EPA, but not arachidonic acid (ARA, C20:4n-6), promoted beige adipocyte formation from primary *h*ADS [38]. The conversion of uncommitted SV cells into beige fat by fish oil is GPR120 dependent. Treatment with GW9508, a chemical agonist of GPR120, recapitulated the white-to-beige conversion by fish oil [21]. Conversely, beige conversion by EPA was abolished in SV cells prepared from GPR120 null mice or in the presence of AH7614, a chemical antagonist of GPR120 [21]. However, GPR120 activation by EPA in fully-mature white adipocytes does not cause beige conversion, suggesting that EPA acts on beige adipocyte recruitment rather than by promoting trans-differentiation [37].

The involvement of GPR120 in beige adipocyte differentiation was further confirmed in animal studies. Quesada-Lopez et al. demonstrated that feeding adult mice with GW9508 for 7 days, significantly upregulated thermogenic genes (*i.e., Ucp1, Pgc1a, Fgf21, Sirt3*), and caused browning of inguinal WAT (iWAT) [21]. The simultaneous administration of CL316243, a β3-specific adrenergic receptor agonist, with GW9508 showed a synergistic increase in oxygen consumption rate and browning of WAT. In contrast, genetic ablation of GPR120 completely abolished cold- and β3 agonist-induced WAT browning. This particular study demonstrated that elevated levels of FGF21 are linked with GPR120 activation in both WAT and BAT, suggesting a novel signaling axis in which adipocyte GPR120 links with FGF21, a key endocrine hormone for fatty acid oxidation [21]. Consistent with this study, TUG-891, another GPR120-specific agonist, significantly promotes fat oxidation and adipocyte browning through Gq/α-mediated calcium release, mitochondrial depolarization, and mitochondrial fission [39].

In summary, multiple pathways work in concert to facilitate beige fat induction through GPR120 activation via either dietary n-3 PUFA supplementation or by chemical agonism (Figure 1B).

C. Regulation of innate immunity by n-3 PUFA in beige fat differentiation— White adipose tissue is an important endocrine organ releasing numerous adipokines that can alter inflammatory status and insulin sensitivity. Adipose tissue inflammation is mediated by the inflammatory responses of adipose tissue macrophages (ATMs), Oh et al. demonstrated the anti-inflammatory function of GPR120 in adipose tissue [34]. Activation of GPR120 by n-3 PUFA or chemical agonists preferentially promotes anti-inflammatory M2 macrophage polarization and protects from HF diet-induced metabolic dysfunction [34]. In the context of WAT browning, the physiological relevance of type 2 innate immune responses has been recently highlighted [40]. ADRB3 pathway activation by cold stress initiates type 2 innate immune responses and alternative M2 macrophage polarization, which mediates UCP1-positive beige fat development. M2 macrophages have been shown to provide local catecholamine within WAT. In addition, groups of type 2 innate lymphoid cells (ILC2s) and eosinophils are the major source of type 2 cytokines IL-33, IL-4, and IL-13 that are necessary for proliferation and commitment of beige precursor cells into beige adipocytes [41, 42]. Despite numerous indications, a missing link remains as to whether fish oil supplementation (or GPR120 activation itself) amends the immunological makeup of WAT into a favorable state for proliferation of beige progenitor cells.

New evidence suggests FGF21 is an important mediator for type 2 innate immune responses in WAT. Huang et al. reported that FGF21 acts on adipocytes in an autocrine manner to promote the production of CCL11, which subsequently promotes eosinophil recruitment, IL-4 release, M2 macrophage polarization, and proliferation and differentiation of beige progenitor cells into thermogenic beige fat cells [43]. Consistently, Quesada-Lopez et al. have demonstrated that GPR120 activation failed to induce beige fat induction in FGF21 null mice and vice versa [21], suggesting that a GPR120/FGF21 axis is essential for WAT browning. Given the indispensable function of GPR120 on FGF21 production, it is likely that fish oil-induced beige fat may trigger type 2 innate immune responses and eosinophil recruitment in WAT. To validate this notion, it is necessary to evaluate the signaling relay of FGF21/CCL11/type 2 lymphoid cells proposed by Huang et al. [43] in GPR120 null mice.

In summary, the activation of GPR120 in inguinal adipose tissue mediates the production of FGF21, resulting in immunological remodeling of adipose tissue such as recruitment of type 2 innate immune responses, recruitment of eosinophils, ILC2s and M2 macrophages, and proliferation of beige precursor cells. (Figure 1B).

2.2. Thermogenic activation by fish oil through GPR120-independent mechanisms

A. Thermogenic activation by n-3 PUFA through TRPV1—N-3 PUFA supplementation triggers multiple signaling pathways for thermogenic reprogramming, which GPR120 activation alone cannot explain. Activation of the sympathetic nervous system (SNS) plays a critical role in non-shivering thermogenesis by innervating BAT and, to a lesser extent, WAT. Detected in the skin, cold is a strong afferent signal to stimulate the SNS in the hypothalamus. Stimulation of the SNS induces efferent signaling acting upon BAT and WAT to produce catecholamines, which activate β3-adrenergic receptors (ADRB3), produce cAMP, and thereby triggers the thermogenic program [44]. Though the sympathetic outflow signaling to BAT/WAT could be identical, the afferent signal to SNS by food-borne molecules originates from temperature-sensing mechanism in the gut [45, 46]. An example of a dietary component is capsaicin, the major pungent component in hot red peppers. Dr. Saito's group has extensively investigated the ability of capsaicin (or capsinoids) to bind to transient receptor potential vanilloid 1 (TRPV1), a nonselective calcium channel located on peripheral sensory neurons in the gut, sending out a thermogenic stimulus to the SNS [4, 47, 48].

Similar to capsaicin, fish oil induces brown/beige fat through activation of the SNS. Kim et al. demonstrated that fish oil supplementation for 10 weeks increased oxygen consumption and core body temperature in mice [20], which was abolished by propranolol, a potent β-blocker. Consistently, elevated levels of levels of cAMP in serum and peripheral tissue after fish oil consumption was reported [19, 20]. The gut-brain-adipose tissue axis seems to be essential, as evidenced by browning effects were abolished in mice with the removal of the vagal nerve, vagotomy, or genetic deletion of TRPV1 [20]. In agreement with this notion, n-3 PUFAs are well-known ligands for TRPV1 [49]. Ohyama et al. proposed new evidence that capsaicin-mediated TRPV1/SNS axis activation may stimulate β2-adrenoceptors (ADRB2) in white fat rather than the β3-adrenoceptor. Furthermore, this study demonstrated that synergistic SNS activation by sub-ambient temperature (17°C) and TRPV1 activation in

the gut by capsaicin strongly enhanced WAT browning, resulting in > 2-fold weight loss relative to that caused by cold or capsaicin alone [50].

Collectively, these studies suggest that n-3 PUFA, independent of GPR120-mediated signaling, serve as a stimulator for TRPV1 in the gut, activating the SNS to trigger ADRB3-mediated signaling cascades in both BAT and WAT (Figure 2A). It is worth testing whether the combined strategy of chronic sub-ambient temperature and dietary regimen with fish oil will constitute a practical approach to promote brown/beige thermogenesis in humans.

B. Impact of n-3 PUFA-driven oxylipins on thermogenic activation of beige adipocytes—The Western diet is deficient in n-3 fatty acids (n-6/n-3=15~17), and a high ratio of dietary n-6/n-3 is associated with increased risk of various metabolic diseases including obesity [51]. Pisani et al. first addressed the impact of n-6/n-3 ratio on beige fat conversion [52]. By using *h*ADS, it has been shown that n-6 PUFA, *i.e.,* linoleic acid (LA, C18:2n-6) and arachidonic acid (ARA, C20:4n-6), strongly inhibit adipocyte browning. This inhibitory effect was associated with ARA-derived eicosanoids of PGE2 and PGF2 via cyclooxygenase (COX) activity [52]. Ghandour et al. have compared the effective thermogenic potential of an n-6 enriched diet (n-6/n-3=30) composed of linoleic acid (LA, C18:2n-6) and oleic acid (OA, C18:1n-6) with an n-3 supplemented diet (n-6/n-3=3.7) mostly composed of alpha-linolenic acid (ALA, C18:n-3) [53]. This study revealed that n-2 prostaglandin series derived from n-6 PUFA, *i.e.*, PGF2 and PGE2, were inhibitors of adaptive thermogenesis. More importantly, it was proposed that a high intake of n-3 PUFA and its oxygenated lipid mediator prostacyclin (PGI2) promotes beige fat formation by suppressing PGE2 and PGF2 production in adipocytes, implicating the competition between n-3 vs. n-6 FA for COX activities [53]. In accord with this idea, PGI2 or a stable analog of prostacyclin promotes browning of *h*ADS through mechanisms associated with cognate receptor IP-R/cAMP signaling and upregulation of PPARγ [54]. Taken together, these studies suggest that the n-6/n-3 ratio *per se* is an important modulator for white-to-beige thermogenic conversion through oxygenated lipid mediators (oxylipins) independent of GPR120-mediated signaling cascades (Figure 2B).

2.3. Current knowledge, controversies, and directions for future research

We summarized recent studies that investigated the role of n-3 PUFA in influencing thermogenic function in animals and relevant cell models of brown and beige adipocytes (Table 1). n-3 PUFA possess unique properties to promote brown/beige thermogenesis compared to other long-chain saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), or n-6 PUFA [19, 38]. This agrees with literature suggesting that n-3 PUFA are better ligands for GPR120 than n-6 PUFA [22]. Moreover, these data are consistent with recent results that metabolites of n-3 PUFA are much more potent agonists for the TRPV1 receptor than metabolites of ARA [55]. N-3 PUFA seemingly exhibit distinctive mechanisms on brown vs. beige precursor cells, although GPR120 is involved in both brown and beige fat development. Based on literature, we suggest a molecular framework that n-3 PUFA act on brown precursor cells at the transcriptional level during brown adipogenesis (Figure 1A), whereas n-3 PUFA alter the microenvironment of WAT at the level of recruitment and proliferation of beige precursors (Figure 1B). In agreement with this current

hypothesis, trans-differentiation of white to beige adipocytes was not triggered by n-3 PUFA [37]. Herein, we categorized ADRB3 activation by n-3 PUFA as a GPR120-independent pathway for convenience's sake. Given that adaptive thermogenesis is an integrative response linking the central nervous system to peripheral adipose tissues, the activation of sympathetic neurons by n-3 PUFA can be regarded as a concurrent and synergistic signal parallel to the GPR120-dependent peripheral responses rather than exclusive to each other.

One important but unanswered question is the thermogenic effectiveness among the different dietary n-3 PUFA, *i.e.,* DHA, EPA, ALA or docosapentaenoic acid (DPA, C22:5n-3). To date, most studies tested the thermogenic function of n-3 PUFA by using fish oil that contains mixtures of EPA and DHA, and no study was conducted to evaluate the thermogenic function of ALA or DPA alone. Despite some controversies, EPA seems to be a stronger thermogenic stimulus than DHA in general, based on studies on murine brown adipocytes or *h*ADS [19]. One reasonable answer is found in studies conducted by Dr. Pisani's group. A fascinating hypothesis is proposed that oxylipins derived from different PUFA are downstream effectors to modulate thermogenic remodeling of beige fat. In particular, Pisani et al. claimed that PGI2 is a pro-thermogenic eicosanoid wherein EPA is a better substrate of COX for PGI2 production rather than DHA [56]. Conversely, it is suggested that DHA is insufficient to compete with ARA and less effective in suppressing anti-adipogneic oxylipin production of PGF2 and PGE2, although DHA features a stronger effect on inhibiting COX2 activity. This notion seems to align with the several cohort studies demonstrating EPA, but not DHA, is effective in reducing triglyceride levels in humans [57, 58]. However, the likelihood of the impact of n-6/n-3 ratio on beige fat development warrants additional support from other researchers, as all studies were published from a single group [56–58]. Also, it is uncertain how competition between n-3 vs. n-6 PUFA toward COX activity affects recruitment of beige precursor or the immunological setting of WAT. Further research is required to test these hypotheses in a relevant system. For example, use of the transgenic fat-1 mouse that converts n-6 PUFA into n-3 endogenously would be a useful model system to investigate the function of a low n-6/n-3 ratio in regulating adaptive thermogenesis probably without the influence of GPR120 signaling. Moreover, future studies to distinguish the thermogenic function of plant-based n-3 PUFA, e.g., flaxseed oil or nut oil (rich in ALA), from marine-based fish oil (rich in DHA and EPA) would be essential to establish a solid role of a low n-6/n-3 ratio in regulating adaptive thermogenesis.

There are some caveats since most cell-based studies used approximately 100–200 μM of EPA, which may not be attainable by oral intake of a fish oil-containing diet or pills. Nonetheless, these studies suggest a new research avenue that n-3 PUFA-enriched diets possess the potential to alter the fate of adipogenic stem cells into mitochondria loaded, fat-burning beige adipocytes rather than fat-depositing white adipocytes.

3. Human clinical studies supporting the thermogenic function of n-3 PUFA

The physiological relevance of brown/beige fat in obesity has been well established in humans [59]. It is estimated that healthy adults contain upward of ~60 g of brown/beige fat (<0.1% of body weight), which could be responsible for >20 % of daily energy expenditure [60]. Unfortunately, induction of thermogenic fat in response to cold exposure is severely

compromised in obese individuals [61, 62]. Conversely, loss of thermogenic activity, whether classical brown or beige fat, contributes to obesity. There are numerous studies supporting that n-3 PUFA decreased inflammation and improved insulin sensitivity in metabolically unhealthy humans [63–69]. However, the implication of thermogenic function of n-3 PUFA is less evident in human clinical trials, despite the clear indication in the aforementioned animal studies. This is likely due to technical difficulties in measuring thermogenic energy expenditure in humans; thus, few studies were designed to assess thermogenic heat release or energy expenditure. In this section, we revisit some of the previous human clinical trials showing that n-3 PUFA affect metabolic rates and adiposity, which may help us provide insight into the thermogenic function of n-3 PUFA in humans.

3.1. Revisiting the clinical evidence on the thermogenic function of n-3 PUFA

One of the most noticeable effects of n-3 FA supplementation is reduced fat mass. Based on the meta-analysis involving 11 randomized clinical trials, Zhang et al. revealed that n-3 PUFA intake significantly reduced serum levels of triglyceride and waist circumference without affecting body mass index (BMI) [70]. Results reported from this study confirm the potential function of n-3 PUFA on reducing visceral fat. However, conclusions from this report are uncertain due to limitations, such as small-scale randomization and poor quality control. The fat loss effect by fish oil was also noted in a study of insulin-resistant adults conducted by Ramel et al [71]. A total of 324 participants aged 20–40 years with BMI 27.5–32.5 were randomly assigned 0–2.1g/day of n-3 PUFA for 8 weeks. This study revealed that 2.1g/day n-3 PUFA was linked to a significant decrease in body weight, plasma levels of fasting insulin, glucose and triglyceride, and improved insulin sensitivity [71]. However, these studies were not designed to understand the underlying mechanisms, and thus it is difficult to infer a thermogenic function of fish oil solely due to reduced adiposity.

The very first crossover-study to determine the effects of n-3 PUFA on resting metabolic rate (RMR), basal energy expenditure, and body composition was conducted by Couet el al. Supplementation of 6g/day of fish oil for three weeks resulted in reduced body fat mass. It also decreased respiratory exchange ratio (RER), indicating increased fuel usage from fat, and increased basal lipid oxidation without altering resting metabolic rate [72]. This study posed several limitations such as small size (only six participants), gender imbalance (5 males and one female), and seasonal differences between two cohorts. Noreen et al. conducted another study to determine the oxidative potential after fish oil intake [73]. In this study, 6 weeks of fish oil supplementation (4g/day) significantly increased lean body mass and reduced fat mass in healthy adults (total of 44 men and 34 women), although significant differences in basal metabolic rate (BMR) or RER were not observed [73]. In parallel with these results, the inclusion of fish or fish oil in randomized trials of weight-loss-diets offered increased weight loss in healthy humans [74, 75]. Most recently, Jannas-Vela et al. reported an interesting study [76] that determined the BMR and substrate oxidation in young healthy males subjects after 12 weeks of fish oil supplementation (2g/d EPA, 1g/d DHA) in comparison to olive oil intake. The authors identified that fish oil intake increased fatty acid and carbohydrate oxidation in the winter season, but not in summer, regardless of RMR. These results imply that fish oil-mediated thermogenic activation may require additional environmental stimuli, such as cold temperature [76]. In the same context, the effects of n-3

PUFA were augmented with exercise [77], another signaling factor to promote adaptive thermogenesis [78]. Another important aspect to consider is gender differences in WAT browning. In contrast to the study performed by Jannas-Vela et al. [76], Logan et al. reported that 12 weeks of fish oil supplementation (3g/day) significantly increased resting BMR as well as exercise-induced fat oxidation in females [79]. This result is also consistent with the finding that women contain a detectably higher amount of brown fat mass than males [80].

3.2. Limitations of studies and directions for future research

In Table 2, we summarized the clinical trials discussed in this review. The thermogenic effects of n-3 PUFA in human clinical trials, mostly fish oil supplementation, remain inconclusive, despite a strong correlation between fish oil intake and reduced visceral adiposity. The inconsistency between animal studies and human clinical trials seems to originate from the confounding factors of study design and technical difficulties in brown fat identification in humans. Unlike experimental design for rodents, direct stimulation of ADRB3 via pharmacological agonists or chronic exposure to suboptimal low temperature is not appropriate for human clinical trials. Hence, better study designs are required to address the thermogenic function of n-3 PUFA intake in adaptive thermogenesis in humans. In agreement with this notion, the reduced adiposity by fish oil supplementation seems to become increasingly evident with additional signaling cues for thermogenesis such as calorie restriction, cold temperature, and exercise [74–76]. Therefore, fish oil supplementation in combination with other lifestyle modifications could be a better strategy to promote adaptive thermogenesis. This hypothesis needs further research with well-controlled and large-scale human trials in both genders. In addition, direct evidence such as ^{18}F-2-deoxy-glucose (FDG) uptake using positron emission tomography (PET)-scans should be provided to establish stronger links with adaptive thermogenesis in humans [81].

4. Perspective of n-3 PUFA supplementation on thermogenic activity in different life stages

In this section, we would like to discuss the physiological perspective of n-3 PUFA supplementation on brown/beige fat activity at different life stages of pregnancy and senescence.

4.1. Prenatal exposure of n-3 PUFA, BAT development, and childhood obesity

Pregnancy, especially in the late gestation period, is the critical window of time for fetal growth including classical BAT development. Augmentation of classical BAT development at the time of birth and increased retention in childhood could be a promising intervention strategy to counteract obesity and metabolic syndrome. Until now, several non-nutritional factors have been shown to increase the development of BAT formation in the fetus. These include thyroid hormone, catecholamines [82, 83] or maternal cold exposure [84], suggesting that activation of maternal sympathetic innervation promotes ADRB3 signaling in the fetus, thereby enhancing the development of BAT. Currently, there is growing information that maternal or infant nutritional status alters BAT mass/activity in newborns and its maintenance in adulthood. Malnutrition at late gestational phases, such as low protein intake [85–87] or HF diet [88], results in reduced BAT development in animal models.

Maternal supplementation of n-3 PUFA increases the n-3 PUFA concentration in the placenta and uterus cord blood, suggesting that n-3 FA are effectively transferred to the fetus in utero [89]. Pregnant women are recommended to take 600~800 mg of n-3 PUFA daily during pregnancy, and no adverse effects were found with up to 2.8 g/day of n-3 PUFA at the late gestation period until delivery [90]. Several human studies report a negative correlation between maternal n-3 PUFA intake and prevalence of childhood obesity [91–94]. In a large population-based cohort study with 4830 mothers, Vidakovic et al. showed that higher maternal n-6/n-3 ratio was correlated with higher risk of childhood obesity [91]. In another prospective cohort study, Moon et al. identified that maternal n-3 FA intake was associated with offspring lean body mass in 12,583 participants [93]. More interestingly, a long-term follow-up study showed that fish oil supplementation during pregnancy in obese mothers had long-lasting effects on reduced adiposity in their offspring [94]. Intriguingly, a recent study by Rudolph et al. supports this model demonstrating that low perinatal n-6/n-3 ratio serves as a metabolic cue to attenuate the susceptibility against diet-induced obesity in adult offspring [95].

Based on these results, it is conceivable that maternal fish oil intake may promote prenatal BAT reprogramming via the aforementioned mechanisms found in section 2.1 or may delay the postnatal degeneration of BAT in early life (Figure 3A). Our group is currently undertaking a pilot study to address the metabolic benefit of maternal n-3 PUFA nutrition on fetal BAT development in animals. While our results are promising, this possibility is still to be reported in the primary literature. In addition, the broader implications of maternal n-3 PUFA supplementation on gestational diabetes, obesity outcomes, and BAT activity in mothers in addition to infants remains to be determined in the context of thermogenic activation.

4.2. n-3 PUFA supplementation and aging-mediated reduction in thermogenesis

The probability to detect brown/beige adipose tissue is inversely correlated with age, implying that thermogenic potential declines with senescence [80]. Understanding the exact cause and identifying an intervention strategy to revert this thermogenic reduction have strong clinical implications for improving metabolic health. Berry et al. have found that old mice (1-year-old) failed to activate cold-induced beige thermogenesis compared to young mice (2-month-old) [96]. They identified that 1) cellular aging of beige progenitors is driven by cell cycle inhibitors (i.e., $p21^{cip}$ and $p16^{Ink4a}$, and $p19^{Arf}$) and stress-activated kinases p38, and 2) senescent beige precursors are unable to proliferate and differentiate upon cold stimulus. Blockade of cellular senescence by genetic deletion of Ink4a/Arf or a small molecule inhibitor of p38 reversed the aging-mediated decrease in thermogenesis [96].

Based on the proposed mechanism above, dietary molecules that possess the ability to suppress cellular senescence of beige precursor cells could be effective in rejuvenating WAT-browning with aging. Compared to other health benefits, the anti-aging function of n-3 PUFA is less clear and poorly understood [97]. Although the exact mechanism was not presented, several clinical trials have reported anti-aging effects of n-3 PUFA; marine n-3 PUFA intake (DHA+EPA) was associated with significant attenuation of telomere shortening in patients with coronary artery disease [98] and elderly individuals [99].

Regarding this mechanism, Chen et al. revealed that fish oil supplementation suppressed aging-mediated reduced-telomere activity by maintaining redox homeostasis; DHA intake was effective in suppressing overexpression of p16 and p53, which are metabolic culprits to promote cellular senescence [100]. Supporting this concept, fish oil supplementation improved energy expenditure and promoted RMR in elderly females [79]. It remains to be determined whether the proposed axis activation of 'redox-telomere-cell cycle inhibition' will be relevant to beige precursor cells (Figure 3B).

4.3. Limitations and directions for future research

We have discussed the basic promise of n-3 PUFA supplementation as a lifetime stimulator for brown/beige fat. This offers the possibility that n-3 PUFA intake exerts inter-generational metabolic benefits through fetal BAT development, and reverses age-related metabolic slowdown by suppressing telomere shortening in beige precursor cells. In fact, there is a paucity of evidence to support these hypotheses to date. Hence, the impact of prenatal n-3 PUFA exposure on fetal BAT development, and its metabolic ramifications in diet-induced obesity should be assessed before implementing maternal n-3 PUFA as a realistic and long-lasting therapeutic target to mitigate childhood obesity. To achieve this aim, randomized clinical trials are required with careful consideration of several factors such as supplementation periods, dose, the source of n-3 PUFA, and the ratio of DHA to EPA. Similarly, innovative study designs are required to distinguish metabolic improvement due to adipose tissue browning from other n-3 PUFA related metabolic benefits in elderly. In addition, rejuvenating WAT browning in the elderly poses a challenge since it demands two separate signaling cues, suppression of cellular senescence and activation of ADRB3 signaling. It will be of great interest to determine whether n-3 PUFA supplementation in the elderly can exert a coordinated role in inhibiting cellular senescence as well as stimulating ADRB3 for WAT browning.

For future research, the emerging role of n-3 PUFA as an epigenetic modulator [19, 101] needs to be counted as a contributing mechanism for maternal nutrition. Intriguingly, there is a challenging hypothesis that requires our attention. Hasegawa et al. demonstrated PRDM16-mediated adipocyte browning is inversely associated with adipocyte fibrosis [102]. It will be exciting to identify whether n-3 PUFA promotes beige fat activation by attenuating aging-mediated fibrosis. Lastly, the role of n-3 PUFA in UCP1-independent thermogenesis in beige adipocytes would be another fascinating aspect to consider. Recently Ikeda et al. reported that calcium cycling in beige adipocytes elevates energy expenditure in the absence of UCP1 [103]. Supported by work showing GPR120 agonism promotes thermogenesis via calcium influx-mediated mitochondrial fission independent of UCP1 activity [39], exploration of calcium signaling-dependent thermogenesis by n-3 PUFA will be of interest, especially in older adults whose UCP1 activation is compromised.

5. Conclusion

The metabolic role of brown/beige fat activity in humans has gained great attention during the last decade. The current review highlights the molecular networks that n-3 PUFA can serve as a safe source of dietary molecules to promote adaptive thermogenesis via GPR-120

dependent or independent mechanisms (Figure 1 and 2). To date, our current understanding regarding the thermogenic function of n-3 PUFA in humans is inconsistent, despite numerous indications in experimental animals. The existing discrepancy between animal studies and human clinical trials is expected to reconcile in the near future provided that FDG-PET becomes accessible to human clinical trials with improved resolution of PET imaging.

Supported by literature, it is conceivable that n-3 PUFA supplementation at targeted-lifetime stages such as pregnancy or senescence could promote brown and beige fat activity, counteracting childhood obesity or aging-associated metabolic slowdown, respectively (Figure 3). It also reinforces the importance of future work to determine other factors that regulate the collective efficacy of thermogenic function of n-3 PUFA such as supplementation dose, period, and EPA/DHA ratio in human clinical trials.

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Abbreviations used

ADRB3	beta 3-adrenergic receptor
ARA	arachidonic acid
ALA	alpha-linolenic acid
BAT	brown adipose tissue
BMR	basal metabolic rate
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FGF21	fibroblast growth factor 21
GPR120	G-protein-coupled receptor 120
***h*ADS**	human adipose-derived stem cells
HF	high fat
iBAT	interscapular BAT
miRNAs	microRNAs
MUFA	monounsaturated fatty acids
PUFA	polyunsaturated fatty acids
RER	respiratory exchange ratio
SFA	saturated fatty acids

SV	stromal vascular
SNS	sympathetic nervous system
TRPV1	transient receptor potential vanilloid 1
UCP1	uncoupling protein 1
WAT	white adipose tissue)

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Highlights

- GPR120 is a signaling platform for adaptive thermogenesis by n-3 PUFA
- n-3 PUFA induce endocrine-immune interactions for beige fat differentiation
- n-3 PUFA activate TRPV1 to trigger ADRB3 signaling for brown/beige fat formation
- A low n-6/n-3 ratio in adipocytes promote white to beige adipocyte conversion.
- n-3 PUFA intake in pregnancy and senescence may promote adaptive thermogenesis

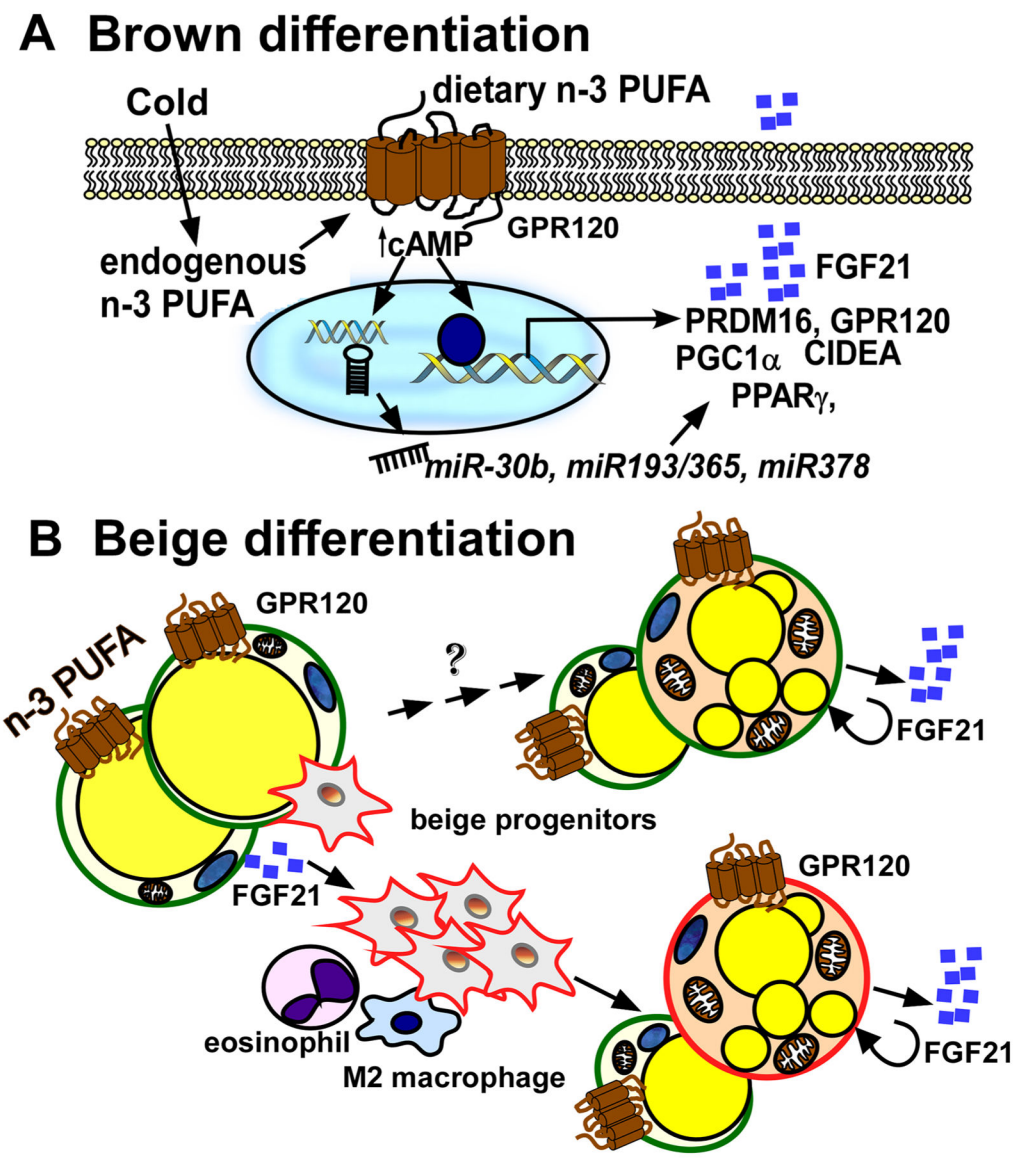

Figure 1. Activation of brown and beige thermogenesis by n-3 PUFA by GPR120-dependent mechanisms

A. Cold exposure increases endogenous production of n-3 PFUA and GPR120 transcription. Recognized by the GRP120 receptor, n-3 PUFA, either endogenously formed or obtained by dietary intake, trigger signals to potentiate brown thermogenesis in precursor cells through cAMP/miRNA-dependent (miR30b, 193b/365, miR378) transcriptional reprogramming in BAT. FGF21 secretion is augmented by n-3 PUFA in BAT, increasing thermogenic activity. **B**. In the WAT, the activation of GPR120 by n-3 PUFA increases FGF21 secretion. The autocrine and paracrine effects of FGF21 alter adipose immune responses including recruitment of M2 innate immune responses (*e.g.*, eosinophil and M2 macrophage polarization) and proliferation of beige precursors (red). These collective modifications of endocrine and immune function by n-3 PUFA promote beige adipocyte differentiation from precursor cells rather than trans-differentiation of white adipocytes (green).

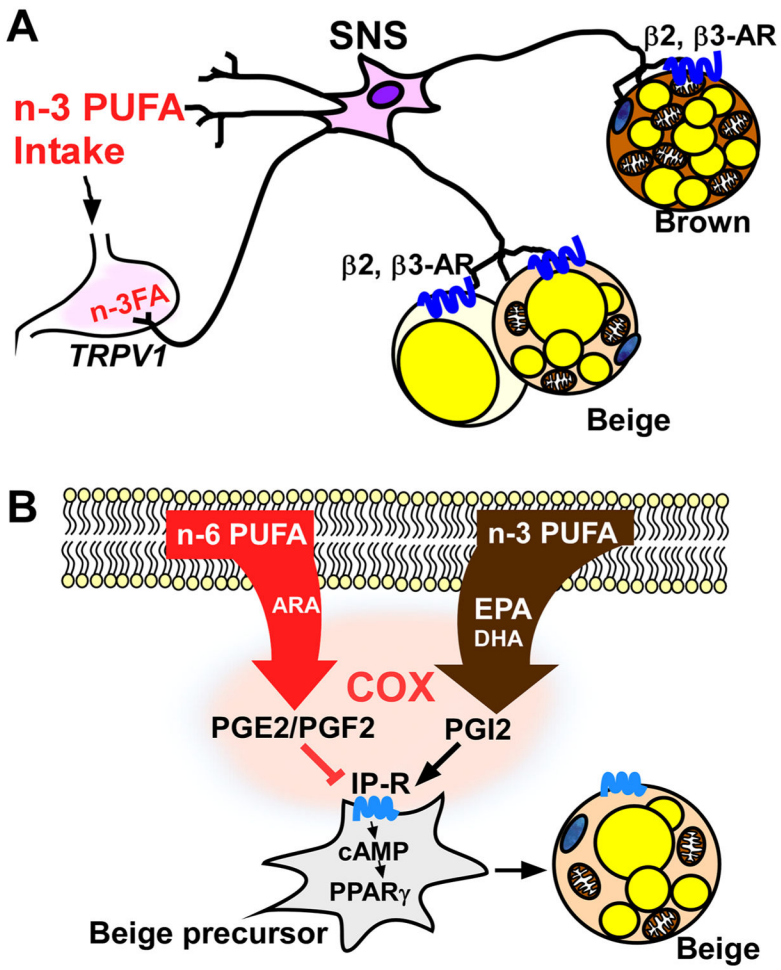

Figure 2. Activation of brown and beige thermogenesis by n-3 PUFA by GPR120-independent mechanisms

A. n-3 PUFA serve as ligands of the TRPV1 receptor in the gut, activating the sympathetic nervous system (SNS), which in turn triggers the ADRB2- or ADRB3-mediated signaling cascade for thermogenic activation in both brown and beige adipocytes. **B.**A high n-6/n-3 ratio is linked with ARA mediated anti-adipogenic oxylipin production of PGE2 or PGF2 inhibiting beige fat differentiation. In contrast, a low n-6/n-3 ratio in adipocytes increases thermogenic oxylipin PGI2 production. PGI2 triggers cAMP/PPARγ signaling through its cognate receptor IP-R in beige precursor cells, leading to beige fat induction.

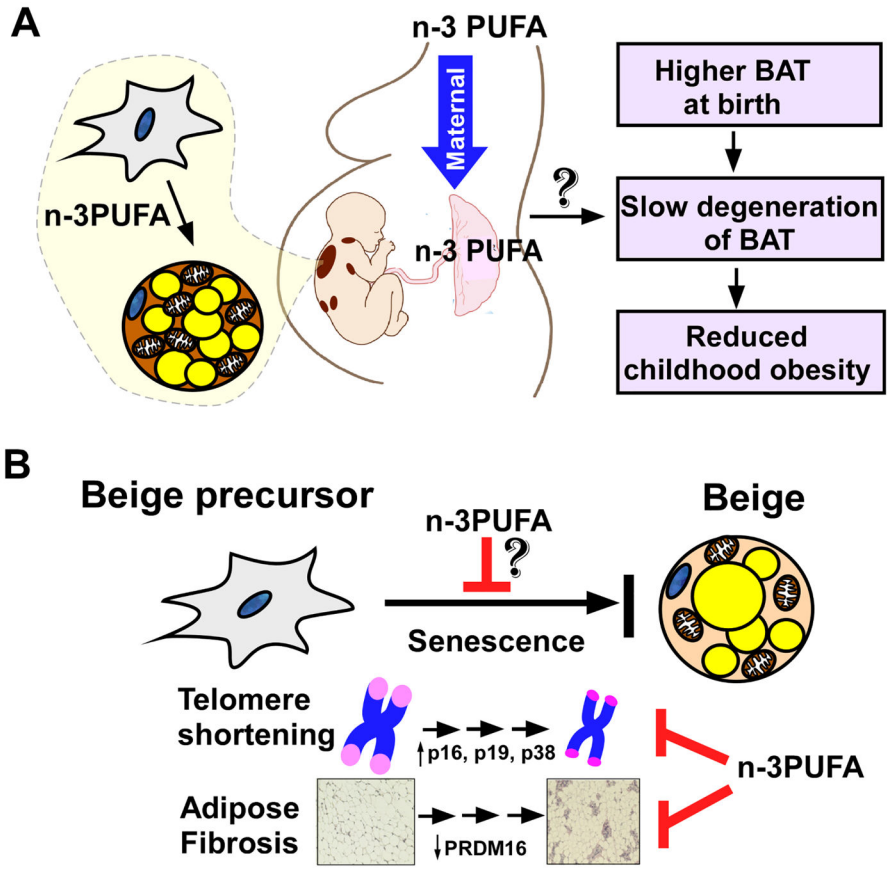

Figure 3. Nutritional perspectives regarding n-3 PUFA intake on thermogenic activity during pregnancy and senescence

A. Maternal intake of n-3 PUFA would be effective in promoting prenatal BAT development across the placenta. The benefits of maternal n-3 PUFA nutrition remain to be determined regarding BAT activity at birth, obesity outcome in childhood and later life. **B.** The decline of beige thermogenic potential with aging is associated with telomere shortening via increased cell cycle inhibitors/oxidative stressors (i.e., p16, p19, p38). Recently, it has also been proposed that adipose tissue fibrosis is inversely linked to adipocyte browning. There are open possibilities that n-3 PUFA supplementation can rejuvenate thermogenesis by interfering with cellular senescence of beige precursors.

Table 1

Thermogenic effects of n-3 PUFA in animals and brown and beige adipocyte models

Test model	Treatment/Dose	Duration	Metabolic Responses	Mechanism	References
iBAT primary mSV	EPA/100μM	5 days	↑ Brown specific gene profile (Ucp1,Cidea, Prdm16, Pparγ) ↑brown specific miRNAs miR-30b, miR193/365, and miR-378	GPR120 ↑ cAMP,	Kim et al. 2016 (19)
C57BL/6 mice	DHA-enriched fish oil (DHA 25%, EPA 8%) EPA-enriched fish oil (EPA 28%, DHA 12%)	10 Weeks	↑ Thermogenesis ↑ Oxygen consumption ↑ Glucose tolerance ↑ Insulin sensitive ↓ Fat mass ↓ body weight.	TRVP1	Kim et.al 2015 (20)
WT GPR120-KO mice FGF21 KO mice	Cold treatment (4 °C) GPR120 agonist GW9508 (50ug/g BW/day)	Cold 24 hours/ GW9508 1wk	GW9508 ↑ brown and beige activity ↑ FGF21 release, which is missing in GPR120 KO mice	GPR120 FGF21secretion	Quesada-López, et al. 2016 (21)
C57BL/6 mice	HF 50% supplementation with 12.5% from EPA and DHA	8 weeks	↓BW ↑glucose tolerance, ↑insulin sensitive, ↑thermogenic markers (beta3-AR, Pgc1α and Ucp1), ↑PPAR (the three isoforms)	beta3-AR	Bargut et al. 2016 (26)
HIB1B cell (murine brown cell line)	EPA100μM	48 hours	↑mRNA expression of Pgc1α and Sirt2 in cell ↑maximum oxidative and peak glycolytic metabolism	↑Glycolysis ↑mitochondrial respiration	Pahlavani et al. 2017 (27)
*h*ADS from overweight females	EPA100–200μM	24 hours	↑ Pgc1α, Sirt1, AMPK	↑ mitochondrial function	Laiglesia et al. 2016 (29)
Primary SV From 129 mice	Cold treatment	10 days	cold treatment ↑ GPR120 expression ~2.5 fold intrinsically	beta3-AR	Rosell et al. 2014 (30)
mSV cells C57BL/6 mice	EPA 200 μM	24 hours	↑ UCP1, CIDEA and VEGFα ↑mitochondrial biogenesis and mtDNA content	↑Mitochondrial biogenesis	Zhao et.al 2014 (37)
*h*ADS from lean women	EPA, AA 20 μM	12 days	EPA ↑ UCP1 and ↑ mitochondrial function. AA ↑ lipid droplet size and ↓ mitochondrial respiratory function.	↑Mitochondrial function	Fleckenstein-Elsen et al. 2016 (38)
C57BL6 mice GPR120 KO mice	GPR120 agonist TUG-891 35mg/kg BW	daily for 2.5 weeks	↓ BW ↓ Fat mass ↑ Fatty acid uptake ↑ Fat oxidation ↓ Lilpid content in BAT and WAT	GPR120	Schilperoor t et. al 2018(39)
iBAT and sWAT Primary mSV Immortalized GPR120 KO brown adipocytes	Vehicle TUG891 with/without GPR120 antagonist AH7614/	30 min	↑ Oxygen consumption ↑ Browning of white adipocytes ↑ Intracellular calcium release	GPR120	Schilperoor t et. al 2018(39)

Test model	Treatment/Dose	Duration	Metabolic Responses	Mechanism	References
*h*ADS cell	ARA 10 μM	3 days	↓UCP1, PPARg-targets ↓ mitochondria activity ↓ Oxygen consumption rate ↑ secretion of PGE2, PGF2α	Oxylipins (PGE2, PGF2α) COX activities	Pisani et. al 2014 (52)
C57BL6 mice	ARA or OA Stimulation with CL316,214 (1 mg/kg/day)	4 weeks	↑PGF2a and PGE2 in sWAT ↓ recruitment of beige adipocytes	n-6/n-3 ratio COX activities	Pisani et. al 2014 (52)
C57BL/6 mice	n6- or n3-supplemented diets (12% energy) CL316,214 stimulation	12 weeks	n3-PUFA ↓ n-6 derived oxylipin production	n-6/n-3 ratio	Ghandour et.al 2018 (53)
*h*ADS	EPA (molar ARA/EPA=3) 10 μM ARA with or w/o 3.3 μM EPA	3 days	EPA ↓ PGF2α and ↑Oxygen consumption	n-6/n-3 ratio	Ghandour et.al 2018(53)
*h*ADS	Carbaprostacyclin (cPGI2)	4 days	cPGI2 ↑ UCP1 mRNA ↑ PPAR signaling	IP-R/cAMP signaling by PGI2	Ghandour et. al 2016 (54)

Abbreviation used: iBAT: interscapular brown adipose tissue, mSV: mice stromal vascular cells, EPA: Eicosapentaenoic acid, UCP1: Uncoupling Protein 1, CIDEA: cell death-inducing DNA fragmentation factor alpha-like effector A, PRDM16: PR domain-containing 16, PPARγ: Peroxisome proliferator-activated receptor gamma, GPR120: G-protein coupled receptor 120, beta3-AR: beta-adrenergic receptor, Sirt2: Sirtuin 2, AMPK: AMP-activated protein kinase, FGF21: Fibroblast growth factor 21, Zic1: Zinc finger of the cerebellum-1, AA: Arachidonic acid, DPA: Docosapentaenoic acid, TRVP1: transient receptor potential vanilloid receptor1, cpdA: compound A (GPR120 agonist), 20-HETE, x-hydroxy ARA: *h*ADS: Human adipose-derived stem cells, PGI2: prostacyclin, cPGI2: carbaprostacyclin (analog of PGI2), COX: cyclooxygenase, PGF2α: Prostaglandin F2α

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Table 2

Human clinical studies showing that n-3 PUFA affect metabolic rates and adiposity

Subject	Treatment	Dose / Duration	Results			References
			ΔBW	ΔFat	Metabolic Responses	
74 psychiatric patients	EPA and placebo plus optional EPA supplementation	Ethyl-EPA 2 g/day 12 weeks double blinded following 40 weeks	-	-	EPA 2 g/day is generally safe when used in patients with schizophrenia for up to 1 year	Emsley et al. 2008 (63)
Insulin-resistant, nondiabetic 34 participants	n-3 EPA Ethyl Esters, placebo	4g/day for 12 weeks	-	↓	n-3 PUFA ↓adipose macrophages, ↓MCP-1 expression ↑n-3 PUFA in adipose tissue and muscle	Spencer et al. 2013 (64)
31 insulin-resistant adults	EPA+DHA or placebo	3.9 g/day / 6 months	-	-	EPA+DHA ↑ hepatic insulin sensitivity	Lalia et al. 2015 (65)
54 T2DM Mexican adults	DHA + EPA-enriched fish-oil, placebo	520 mg / 24 weeks	↓	↓	↓waist circumference, ↑glucose, HbA1c, leptin, leptin/adiponectin ratio, and lipid profile	Jacobo-Cejudo et al. 2017 (66)
60 overweight (BMI > 25), healthy adults, aged 40–60 years	n-3 fatty acid (capsule) probiotic VSL#3 both omega-3 and probiotic	180 mg EPA and 120 mg DHA daily / 6 weeks	-	-	Omega-3 fatty acid ↓ IR, ↓ hsCRP	Rajkumar et al. 2014 (67)
29 subjects (Avg 44 years with MetS)	n-3 PUFA, placebo	2 g/day of omega-3 PUFAs 12 weeks	-	-	↓ IL-6, TG, Cholesterol ↑PAI-1 levels	Tousoulis et al. 2014 (68)
37 patients (50.6±9.8 y) with well-controlled diabetes	Mixture EPA and DHA Corn oil Placebo EPA 2160 mg DHA 1440 mg	48 weeks	-	-	Improved inflammation compared with placebo	Dasarathy et al. 2015 (69)
324 participants (20–40 years, BMI 27.5–32.5, from Iceland, Spain and Ireland)	(30% of total energy) -no seafood (control; 6×500 mg sunflower oil capsules/day); -lean fish (150 g cod, three times/week); -fatty fish (150 g salmon, three times/week); -fish oil capsules (6×500 mg capsules/day)	8 weeks	↓	↓	↓ fasting insulin, glucose, triacylglycerol and adiponectin levels and HOMA-IR ↑ LC n-3 PUFA content in membrane lipids significantly increased (all $p<0.001$)	Ramel et al. 2008 (71)
Six volunteers, 23±2 years, BMI 21.9±1.6	Isocaloric diet (32% fat) Control diet and fish oil diet	6 g/day, 15 weeks	↓	↓	↑ Lipid oxidation	Couet et al. 1997 (72)
A total of 44 participants, 34 ± 13years	4 g/d of Safflower Oil (SO) 4 g/d of Fish oil (FO)	1600 mg/d EPA 800 mg/d DHA for 6 weeks	-	↓	↓ fat mass, ↑ lean mass ↓ salivary cortisol	Noreen et al. 2010 (73)
324 men and women, (20–40 years) BMI 27.5–32.5	(1) control (sunflower oil) (2) cod diet (3x 150 g/week) (3) salmon diet (3x 150 g/week) (4) fish oil (DHA/EPA capsules)	Cod 272 mg n-3 PUFA/d Salmon 3004 mg n-3 PUFA/d Fish oil 1418 mg n-3 PUFA/d / 8 weeks	↓	↓	↓ TG, LDL, TC, greatest in salmon group	Gunnarsdottir et al. 2008 (74) Thorsdottir et.al 2007 (75)

Subject	Treatment	Dose / Duration	Results			References
			ΔBW	ΔFat	Metabolic Responses	
26 healthy males (22.8 ± 2.6 years)	Olive oil capsule Fish oil capsule	3g Olive oil/day 2g EPA, 1g DHA/d / 12 weeks	-	-	↑ Resting FA oxidation no change in RMR	Jannas-Vela et al. 2017 (76)
81 adult volunteers (25–65 years old)	Fish oil (FO), FO and exercise (FOX), Sunflower oil (SO; control), SO and exercise (SOX).	6 g tuna FO/d (1.9 g n-3 PU FA) 6 g SO/d 12 weeks	↓	↓	fish oil intake ↓ TG ↑ HDL cholesterol	Hill et al. 2007 (77)
24 female (66 ± 1 years)	EPA + DHA Placebo	3g/day, 12 weeks	-	↓	FO significantly ↑ RMR ↑EE ↑ Fat oxidation ↑Lean mass ↓TG	Logan et al. 2015 (79)

Abbreviation used: EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, EE: Energy expenditure, n-3 PUFA: Omega-3 polyunsaturated fatty acid, HbA1c: Hemoglobin A1c , T2DM: Type 2 Diabetes Mellitus, HOMA-IR: Homeostatic model assessment - insulin resistance, RMR: Resting metabolic rate, SO: Sunflower oil, FO: Fish oil, MCP-1: Monocyte chemoattractant protein-1, IR: Insulin resistance, hsCRP: High sensitivity C-reactive protein, IL-6: Interleukin 6, TG: Triglyceride, PAI-1: Plasminogen activator inhibitor-1, BMI: Body mass index, MetS: Metabolic syndrome, LC n-3 PUFA: Long chain omega-3 polyunsaturated fatty acid, LDL: Low-density lipoprotein, TC: Total cholesterol, FA: Fatty acid, HDL: High-density lipoprotein