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Of mice and men

Factors abrogating the antiobesity effect of omega-3 fatty acids

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and
Alvheim AR, Malde MK, Osei-Hyiaman D, Hong Lin Y, Pawlosky RJ, Madsen L, et al. Dietary linoleic acid elevates endogenous 2-AG and anandamide and induces obesity. Obesity (Silver Spring) 2012; In press; PMID:22334255; http://dx.doi.org/10.1038/oby.2012.38

The ability of n-3 long chain polyunsaturated fatty acids (PUFAs) to prevent high fat diet-induced obesity in rodents is well documented. Evidence for a similar effect in humans is, however, limited. Intervention studies in humans are inconclusive and epidemiological studies are dichotomous. Our recent finding that sucrose and other high glycemic index carbohydrates abrogate the antiobesity effect of n-3 PUFAs might, at least in part, provide an explanation to the apparent discrepancy between human and rodent intervention studies, and the lack of effect in some human trials. In addition to the amount and type of carbohydrates, the levels of n-6 PUFAs, linoleic acid in particular, in the background diet might influence the antiobesogenic effect of n-3 PUFAs. Lastly, it is plausible that the quantity of persistent organic pollutants in fish oil, and seafood rich in n-3 PUFAs, might have an influence on the outcome of the trials.

A large number of studies have documented the ability of fish oil to attenuate,[1-9] and in at least one case, totally prevent[10] high fat diet-induced obesity in rodents. Moreover, at least one study has demonstrated that n-3 PUFAs are able to reduce the amount of body fat in mice already made obese by a high fat diet.[11] In view of the promising rodent studies one would expect fish oil and seafood enriched in n-3 PUFAs to be a useful, effective and safe tool to reduce obesity also in humans.

Epidemiological associations between intake of n-3 PUFAs and obesity development are, however, inconclusive. Whereas a large prospective cohort study, Nurses' Health Study cohort,[12] reported that higher intake of fish and n-3 PUFAs was associated with higher prevalence of obesity, another US prospective cohort study, The Health Professional Follow-up study, showed that men who consumed fish were less likely to be obese.[13] The latter finding is supported by smaller cross-sectional studies where the level of plasma n-3 fatty acids, used as a biomarker for intake of fish, was inversely correlated with body mass index (BMI) and waist circumfences.[14] Similarly, an inverse relationship has been reported in obese patients between abdominal obesity and amount of n-3 PUFA in adipose tissue samples[15] and between the amount of n-3 PUFAs in subcutaneous adipose tissue and reduced adipocyte size.[16]

Although a few human intervention trials, where weight loss, BMI and/or waist circumstances were reported endpoints, have shown promising results, other similar trials have failed, and taken together the human intervention trials are inconclusive. A systematic review of the subject was published fairly recently by Buckley and Howe,[17] and therefore only a few studies will be mentioned here. The positive and quite convincing results from at least two trials published in 2007 are worth mentioning though. First, Thorsdottir et al.[18] demonstrated that 1.5 g of n-3 PUFA per day during eight weeks of caloric restriction significantly increased weight loss in young overweight men. This was, however, not seen in women. Second, Kabir et al. reported that 3 g of n-3 PUFA per day reduced total fat mass and the diameter of subcutaneous adipocytes in a two month

randomized controlled trial with type 2 diabetic women.[19] However, Krebs et al. did not observe an increased weight loss when 5 g of fish oil was combined with an energy restricted diet in overweight hyperinsulinemic women.[20] Moreover, a meta-analysis from 2009, aimed to investigate the effect of n-3 PUFAs on glycemic control in type 2 diabetic patients, concluded that there was no significant effect on body weight.[21] Several possible explanations for the apparent discrepancy between rodent and humans trials and the lack of consensus in human intervention studies, mostly related to study design, exist and these are discussed in the previously mentioned review by Buckley and Howe.[17] Here, we discuss the findings in three recent rodent studies that provide insight into how interaction between nutrients may, at least in part, explain the discrepancies and inconsistencies observed in human studies.

An important aspect concerning the outcome of human trials concerns the levels of dietary n-6 PUFAs, linoleic acid (LA) in particular, in the background diet. The estimated per capita consumption of soybean oil, containing about 50% LA, has increased more than 1,000-fold from 1909 to 1999 in the US; today representing 7.21 energy percent (e%) of the diet.[22] A recent study performed in collaboration with Dr Alvheim and Dr Hibbeln demonstrated that increasing the dietary levels of LA from 1 to 8 e%, thereby reflecting the increase during the 20th century, elevated the level of arachidonic acid (AA)-phospholipids and promoted obesity in mice.[6] By adding 1 e% n-3 PUFAs to the 8 e% LA diet, EPA and DHA replaced AA in tissue phospholipids, and obesity development was attenuated.[6] The conversion of LA to AA, allows competition between AA and the n-3 PUFAs, EPA and DHA, for incorporation into the phospholipids. Thus, intake of LA will influence the omega-3 index (red blood cell EPA + DHA as a percentage of total red blood cell lipids).[23] It is important to note that there is a large variability not only in intake of n-3 PUFAs but also in LA between different countries, and thus, the omega-3 index is highly variable.[23] Accordingly, the amount of n-3 PUFAs required to meet 50% n-3 PUFAs in tissue is also highly variable. For instance, the average amount to meet this 50% in the Philippines, Denmark and US, is 133, 578 and 2,178 mg per day, respectively.[23] In this respect it is worth noting that we found a strong positive correlation between obesity development and consumption of LA and soybean oil.[6] Also, intake of sugar was positively correlated with obesity, whereas changes in total energy consumption were not.[6] Moreover, intake of calories from poultry, a major source of LA in the US diet,[22] correlated with obesity development, unlike intake of grains, beef, fish and seafood, eggs, dairy or vegetables.[6] In this context, it is worth mentioning that the women in the Nurses' Health Study cohort, who frequently consumed fish and were likely to be obese, also had a high intake of poultry.[12]

A second aspect regarding background diets, when studying the effect of n-3 PUFAs, is the amount and type of carbohydrates. Our recent finding that sucrose[24] and other high glycemic index carbohydrates[25] abrogate the antiobesity effect of n-3 PUFAs in mice, demonstrate that the antiobesogenic potential of n-3 PUFAs is dependent on the macronutrient composition of the background diet. We demonstrated that increasing the sucrose: protein ratio in the background diet, dose-dependently abrogated the antiobesity effect of fish oil.[25] In fact, when the sucrose amount was high, a diet enriched in n-3 PUFAs was as obesogenic as a diet enriched in n-6 PUFAs.[24] A diet containing 35 e% sucrose might surely be of little human relevance. However, we recently demonstrated that inclusion of high glycemic index carbohydrates, such as amylopectin, also abrogated the antiobesity effect of fish oil.[25] Today, grains and sugar represent the major energy sources in the US diet, representing approximately 22 and 17 e%, respectively.[22] If the sucrose:protein ratio, and/or the amount of high glycemic index carbohydrates influence on the antiobesogenic effect of n-3 PUFAs also in humans, both the type and level of carbohydrates in the background diets will affect the outcome of the trials.

Together, these two reports illustrate the importance of a controlled background diet when studying the effect of n-3 PUFAs. The levels of LA and high glycemic index carbohydrates might be of particular importance, as the background levels of LA will determine the amount of n-3 PUFAs required replacing AA in PL, and high glycemic index carbohydrates may reduce the antiobesogenic effect. It is likely that the success and failure of different clinical trials using similar doses of n-3 PUFAs have been influenced by different background diets. The dose of n-3 PUFAs used in clinical trials is generally relatively low, less than 3 g per day, and this might be far too low if the background diet contains high levels of LA.[26]

A third aspect for consideration is the source of n-3 PUFAs and the levels of persistent organic pollutants (POPs) in these. In collaboration with Dr. Ruzzin we have recently demonstrated that whereas inclusion of purified salmon oil attenuated obesity in Wistar rats, inclusion of crude salmon oil exaggerated obesity development.[27] Moreover, we have shown that chronic consumption of Atlantic salmon with a high level of POPs caused obesity in mice.[28] When the levels of POPs in the salmon were reduced, obesity development in the mice decreased concomitantly.[28] POP exposure has been associated to development of type 2 diabetes,[29-33] whereas the correlation between POP exposure and obesity per se has been more difficult to establish. However, a recent Danish cohort study demonstrated a positive correlation between BMI and the concentration of certain POPs, such as dichlorodiphenyltrichloroethane (DDT), in adipose tissue.[34] A major problem linking POPs with obesity development is that plasma levels of POPs are not reliable predictors of POP exposure.[34] Further, since POPs accumulate in adipose tissue, measurements of POP concentrations in plasma and adipose tissue are strongly affected by the increased mass of adipose tissue in obese individuals resulting in a "dilution" of POPs. Thus, a negative correlation between concentrations of polychloridinated biphenyl (PCB) congeners in plasma and BMI has been reported in two separate studies.[35,36] In one of these studies,[35] intake of fish, fatty fish in particular, was associated with the measured levels of adipose tissue POP

concentrations. POPs accumulate in lipid-rich food, and fatty fish thus represent a source of both POPs and n-3 PUFAs. The levels of POPs in farmed fish depend on the composition of the fish feed, whereas contamination in feral fish is determined by factors including fat content, prey and geographic location. Thus, even though a direct causal link between human obesity and POP exposure remains to be established, it seems warranted to consider possible exposure to POPs in human intervention trials where fish or seafood is used as a source of n-3 PUFAs.

Concerning the apparent discrepancy between rodents and humans on the effect of n-3 PUFAs in body weight and obesity it should be mentioned that several rodent studies have reported an effect on adipose tissue weights without a concomitant reduction on body weight.[1,5,7,37,38] Thus, a decrease in body weight in humans might not necessarily be expected. Moreover, two often ignored rodent studies have reported an increased adipose tissue mass in rodents fed n-3 PUFAs. Fish oil is actually reported to increase adipose tissue mass in *db/db* mice when included in a high fat diet[39] and inclusion of 6% menhaden fish oil doubled the amount of adipose tissue mass in female LDL receptor deficient mice, while still preventing hepatic steatosis.[40] Interestingly, no overall effect on adipose tissue mass was observed in a study where both male and female mice were fed a high fat diet supplemented with fish oil in comparison to a high fat diet supplemented with olive oil, whereas an increased adipose tissue mass was still observed in the female mice.[41] This points to a role of the LDL receptor for an intriguing gender dependent difference in fat partitioning and collectively these studies also point to leptin as a key player in the action of n-3 PUFAs. Thus, reduced leptin sensitivity, as often observed in obese subjects, might reduce n-3 PUFA action.

Collectively, the levels of LA as well as the amount and type of carbohydrates in the background diet might influence the antiobesogenic effect of n-3 PUFAs also in humans. Furthermore, the actual content of EPA and DHA varies in different studies, and in combination this may provide an explanation for the apparent lack of effect in some human trials.

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