Low-grade chronic inflammation perpetuated by modern diet as a promoter of obesity and osteoporosis

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Some of the universal characteristics of pre-agricultural hominin diets are strikingly different from the modern human diet. Hominin dietary choices were limited to wild plant and wild animal foods, while the modern diet includes more than 70% of energy consumed from refined sugars, refined vegetable oils, and highly processed cereals and dairy products. The modern diet, with higher intake of fat has also resulted in a higher ratio of omega-6 (n-6) to omega-3 (n-3) polyunsaturated fatty acids (PUFA), contributing to low-grade chronic inflammation (LGCI) and thus promoting the development of many chronic diseases, including obesity and osteoporosis. In this review, we describe the changes in modern diet, focusing on the kind and amount of consumed fat; explain the shortcomings of the modern diet with regard to inflammatory processes; and delineate the reciprocity between adiposity and inflammatory processes, with inflammation being a common link between obesity and osteoporosis. We present the evidence that over-consumption of n-6 PUFA coupled with under-consumption of n-3 PUFA results in LGCI and, along with the increased presence of reactive oxygen species, leads to a shift in mesenchymal stem cells (precursors for both osteoblasts and adipocytes) lineage commitment toward increased adipogenesis and suppressed osteoblastogenesis. In turn, high n-6 to n-3 PUFA ratios in the modern diet, coupled with increased synthesis of pro-inflammatory cytokines due to adiposity, propagate obesity and osteoporosis by increasing or maintaining LGCI.

KEY WORDS: adipocytes; cytokines; eicosanoids; mesenchymal stem cells; omega-3 fatty acids; omega-6 fatty acids; osteoblasts; osteoclasts; polyunsaturated fatty acids

Abbreviations:
5-LOX=5-Lipoxygenase; AA=Arachidonic acid; AHA=American Heart Association; ALA=Alpha-linoleic acid; CLA=Conjugated Linolenic Acid; COX-2=Cyclooxygenase-2; CRP=C-reactive protein; DHA=Docosahexanoic Acid; EPA=Eicosapentanoic Acid; IGBP-3=Insulin like growth factor binding protein-3; IL-1=Interleukin-1; IL-6=Interleukin-6; LA=Linolenic Acid; LBT4=Leukotriene B4; LGCI=Low grade chronic inflammation; MSC=Mesenchymal stem cells; MUFA=Monounsaturated Fatty Acids; n-3=Omega-3; n-6=Omega-6; NHANES=National Health and Nutrition Examination Survey; OPG=Osteoprotegerin; PDE=Phosphodiesterase; PGE2=Prostaglandin E2; PGE3=Prostaglandin E3; PUFA=Polyunsaturated Fatty Acids; RANK=Receptor activator of nuclear factor kappa-B; RANKL=Receptor activator of nuclear factor kappa-B ligand (RANKL); SFA=Saturated Fatty Acids; TNF-α=Tumor Necrosis Factor-alpha; TXA2=Thromboxane A2

Dietary intake and other lifestyle factors (e.g. physical activity, smoking) are environmental modifiers that have changed dramatically with agriculture and animal husbandry, about 10,000 years ago (1, 2). However, a number of anthropological, nutritional, and genetic studies indicate that even more drastic changes happened very recently, some 200 years ago with the onset of industrial revolution, and that these changes could be associated with many chronic diseases or the so-called diseases of civilization (1, 3).

By examining the dietary habits along the evolutionary timeline, one can easily notice the
difference between the pre-agricultural hominine and modern human diet. Hominin dietary choices were limited to wild plant and animal foods. In contrast, 72% of the energy consumed by modern humans includes refined sugars, refined vegetable oils, highly processed cereals and dairy products, as well as high consumption of alcohol (4).

The objectives of this review are to: a) describe the changes in modern diet with regard to several nutrients, particularly addressing the type and amount of consumed fat; b) explain the shortcomings of the modern diet with regard to inflammatory processes; and c) delineate the reciprocity between adiposity and inflammatory processes and the link that connects obesity, osteoporosis, and inflammation. We present the hypothesis that omega-6 (n-6) polyunsaturated fatty acids (PUFA) propagate obesity and osteoporosis by increasing/maintaining low-grade chronic inflammation (LGCI), ultimately shifting the commitment of mesenchymal stem cells (MSC) toward increased adipogenesis.

**Discordance between current and past dietary habits along the evolutionary timeline**

Our genetic makeup has not changed over the last few centuries, but the environment and dietary behaviors have, substantially. The spontaneous mutation rate for nuclear DNA is estimated at 0.5% per million years; thus, very little change has occurred in our genes compared to our Paleolithic ancestors of 40,000 years ago, when our genetic profile was established. Therefore, the profound environmental and dietary changes that began with the introduction of agriculture and animal husbandry some 10,000 years ago, occurred too recently on the evolutionary timeline for the human genome to adapt, contributing to many of the chronic diseases in modern societies (5).

Some nutrients exhibit high discordance regarding their intake in the hominines compared to modern humans that may have contributed to inflammation and subsequent chronic diseases, including obesity and osteoporosis. Of note are the lower consumption of fat, carbohydrates, and sodium (high amounts of the latter might result in negative consequences on bone health via increased calcium excretion) and much higher consumption of calcium and vitamin C (both important in bone health and the former one in weight management). Additionally, with the high intake of plant foods, hominines were consuming much higher amounts of fiber (has a role in weight management) and phytochemicals, known for their anti-oxidative and anti-inflammatory properties.

**Table 1**: Estimated intake of selected nutrients and foods by hominines from the late Paleolithic (~40,000 years ago) and modern USA population and current recommendations

<table>
<thead>
<tr>
<th>Nutrients/ Foods</th>
<th>Late Paleolithic</th>
<th>Current USA Intake</th>
<th>Current USA recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (% of energy)</td>
<td>21</td>
<td>33</td>
<td>20-35</td>
</tr>
<tr>
<td>Carbohydrates (% of energy)</td>
<td>46</td>
<td>50</td>
<td>45-65</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>33</td>
<td>15</td>
<td>10-35</td>
</tr>
<tr>
<td>Alcohol (serving per day)</td>
<td>Minimal</td>
<td>N/A</td>
<td>1-2</td>
</tr>
<tr>
<td>P/S ratio</td>
<td>1.4</td>
<td>0.7</td>
<td>N/A</td>
</tr>
<tr>
<td>Cholesterol (mg per day)</td>
<td>520</td>
<td>261</td>
<td>&lt;300</td>
</tr>
<tr>
<td>Fiber (mg per day)</td>
<td>100-150</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>Sodium (mg per day)</td>
<td>690</td>
<td>3463</td>
<td>&lt;2300</td>
</tr>
<tr>
<td>Calcium (mg per day)</td>
<td>1500-2000</td>
<td>1029</td>
<td>1000-1300</td>
</tr>
<tr>
<td>Vitamin C (mg per day)</td>
<td>440</td>
<td>87</td>
<td>75-95</td>
</tr>
</tbody>
</table>

*Adapted from references (2, 6, 7)*

*P/S - polyunsaturated to saturated fatty acid ratio; N/A - data not available*
wild animals, low in saturated fatty acids (SFA) and high in both PUFA and monounsaturated fatty acids (MUFA), with low n-6 to n-3 PUFA ratio. Prior to the Neolithic (agricultural) period, or some 40,000 years ago in the Paleolithic, all animal foods consumed by hominines were limited to wild animals (9). Approximately 50% of fatty acids in the adipose tissue of wild animals were SFAs, while the dominant type in the muscle and all other organs were PUFA and MUFA. However, for most of the year animals’ subcutaneous and visceral body fat stores were usually depleted, and it was likely that PUFA and MUFA were the predominant fats of the carcass available for hominin consumption. Therefore, high amounts of SFA were probably not available to hominines feeding on these wild animals. Moreover, the consumption of seeds, nuts, various greens, and other wild plants provided the hominines with higher intake of PUFAs and MUFAs and probably with an optimal ratio of n-6 to n-3 (3, 8).

Subsequent changes that occurred with the beginning of animal husbandry and agriculture (in the Neolithic period, some 10,000 years ago) allowed for slaughtering of animals at the peak of body fat accumulation, while the production of grains and other agricultural products with higher SFA content led to an increasing consumption of n-6 PUFAs, creating a less favorable n-6 to n-3 ratio. Technological advances in the early and mid 19th century generated large quantities of grain (especially corn) for animals and humans, which changed the composition of the meat in the cattle, resulting in “marbled meat” characterized by excessive triglyceride accumulation and fat infiltration into the muscular tissue and higher SFA content (10). This and the modernization of food processing and preservation, as well as changes in food choices have altered the ratio between n-6 and n-3 fatty acids in the modern diet and resulted in its low n-3 fatty acid content (8, 9), favoring the development of chronic diseases.

Pro- and anti-inflammatory metabolites of n-6 and n-3 fatty acids

It is known that elevated n-6 PUFA levels stimulate pro-inflammatory processes and promote the pathogenesis of many diseases such as cardiovascular, neurological, autoimmune, as well as cancer, arthritis, and diabetes (3, 8). Recently however, it has been realized that various pro-inflammatory actions may differ.

Table 2 Ratio between n-6 and n-3 polyunsaturated fatty acids in different population groups

<table>
<thead>
<tr>
<th>Population group</th>
<th>n-6/n-3 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paleolithic/Neolithic</td>
<td>0.8-1</td>
</tr>
<tr>
<td>Mediterranean (prior to 1960)</td>
<td>1-2</td>
</tr>
<tr>
<td>Current Northern Europe</td>
<td>15</td>
</tr>
<tr>
<td>Current USA</td>
<td>15-20</td>
</tr>
</tbody>
</table>

Adapted from references (1, 3, 8)

Table 3 Intake of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), the n-6 to n-3 ratio reported by NHANES, and recommendations

<table>
<thead>
<tr>
<th>Intake recommendations</th>
<th>EPA + DHA (g per day)</th>
<th>n-6/n-3 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHANES (2009-2010)</td>
<td>0.12</td>
<td>9:1</td>
</tr>
<tr>
<td>Dietary Guidelines (2010)</td>
<td>0.25</td>
<td>8:1</td>
</tr>
<tr>
<td>AHA (healthy population)</td>
<td>0.5</td>
<td>7:1</td>
</tr>
<tr>
<td>AHA (coronary patients)</td>
<td>1</td>
<td>6:1</td>
</tr>
<tr>
<td>AHA (severe coronary patients)</td>
<td>2-4</td>
<td>3:1</td>
</tr>
<tr>
<td>FDA GRAS level</td>
<td>3</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Adapted from references (1, 3, 8, 11)

NHANES - National Health and Nutrition Examination Survey; AHA - American Heart Association
inflammatory cytokines such as tumor necrosis factor-
and these are associated with resolving the inflammation
specific maresins are the metabolites of DHA (17),
and their action is anti-inflammatory (16). Protectins, the
resolvins, and protectins, also generated by COX-2
prostaglandin E
3), generated by lipid-oxidizing enzyme
inflammatory lipoxins A
4 and B
4 are the anti-inflammatory eicosanoids and include prostaglandin
E
2 (PGE$_2$), leukotriene B$_4$ (LBT$_4$), and thromboxane
A$_2$ (TXA$_2$), generated by lipid-oxidizing enzyme
prostaglandin E$_3$ (PGE$_3$), leukotriene B$_4$ (LBT$_4$), resolvins, and protectins, also generated by COX-2
and 5-LOX. During metabolism, n-6 and n-3 PUFAs
the higher amount of n-6 PUFA in the modern diet
shifts the balance toward the production of pro-
inflammatory eicosanoids (11). Figure 1 illustrates the
metabolic pathways of the n-6 and n-3 PUFA series
in generating various types of eicosanoids.

**Acute and low-grade chronic inflammation and
their resolutions**

The breakdown of fatty acid metabolites and the
conversion of eicosanoids generates different pro- and
anti-inflammatory products (Figure 2). The pro-
inflammatory lipoxins A$_4$ and B$_4$ are the metabolites of AA, and their action is focused on neutrophil
regulation (15), while the E-series resolvins (both E1 and E2) are metabolized from EPA by 5-LOX, and
their action is anti-inflammatory (16). Protectins, the
D-series resolvins (D1 and D2), and the macrophage-specific maresins are the metabolites of DHA (17),
and these are associated with resolving the inflammation
(18). Overall, n-3 PUFA are metabolized into anti-
inflammatory eicosanoids which are important to
resolve inflammation otherwise triggered by any insult
on the cell, ranging from a bruise to a bacterial
infection or even by inflammatory signals from
neighboring cells.

Acute inflammation activates the inflammatory
cascade of prostaglandins, leukotrienes, and other
inflammatory cytokines such as tumor necrosis factor-
alpha (TNF-α), interleukin-6 (IL-6), and interferon-
gamma which recruit neutrophils and macrophages.
When the insult is dealt with, either via apoptosis and
phagocytosis of the infected cells and foreign bodies
or via cleaning the local inflammation, resolvins,
protectins, maresins, and lipoxins all work together to
stop further recruitment of immune cells and the
propagation of pro-inflammatory cytokines, so that
the cell can return to its basal state (19, 20).

In contrast, LGCI is a state of persistent and
unresolved inflammation, where pro- and anti-
inflammatory cytokines are elevated, and inflammation
is not resolved (21). In the clinical context, this state
results from the continuous presence of specific
immune cells leading to 2-4-fold elevations in the
cirulating levels of pro- and anti-inflammatory
cytokines, and more are recruited in a perpetual cycle
(21). The exact PUFA metabolite composition of this
unresolved or surplus inflammation is unknown (17),
but it is likely that there is a bias towards the pro-
inflammatory eicosanoids, particularly with higher
n-6 PUFAs intake (Figure 3).

**The detrimental role of low-grade chronic
inflammation in obesity and osteoporosis**

Obese individuals have abnormal circulating levels
of TNF-α, IL-6, C-reactive protein (C-RP), adiponectin,
and leptin, as these molecules are overexpressed in
adipose tissue (22-24). PGE$_2$, a metabolite of AA, has
been found to mediate locally the biological action of
TNF-α and Interleukin-1 (IL-1) in the cases of fever
and local inflammation. Leptin and adiponectin are
adipokines produced by adipose tissue that mediate
chronic inflammation. Leptin has been found to
stimulate inflammatory responses (25, 26), while
adiponectin acts as an anti-inflammatory adipokine
(27). The constant presence of these inflammatory
cytokines associated with increased adiposity
promotes persistent LGCI in obese individuals (1),
while adipose tissue itself increases their production,
resulting in a perpetual feedback cycle from one to
another (adiposity↔inflammation). For more
extensive review see Kelly et al. (11).

In the bone, inflammation also plays a detrimental
role and is interconnected with adiposity. It is well
established that IL-6 stimulates osteoclastogenesis
formation of bone-resorbing cells) and is considered
an osteocorsptive factor (28). Simultaneously, higher
amount of fat mass activates osteoclasts via increase
in other cytokines like IL-1 and TNF-α, as shown in
Figure 4. Osteoclasts are derived from the same
hematopoietic stem cells as the immune cells (29). In LGCI accompanied by increased T-cell activation, osteoclastogenesis and bone resorption are accelerated, as the osteoclasts are programmed to respond to inflammatory signals. Therefore, in states such as LGCI, osteoclastogenesis and bone resorption are increased (11), leading to a perpetual feedback cycle (bone resorption ↔ inflammation).

Mesenchymal stem cells (MSC) are the common precursors for osteoblasts (bone forming cells) and adipocytes (30). However, inflammation may deregulate the MSC lineage commitment (11, 31). Even under normal homeostatic conditions, MSC slightly favors adipogenic differentiation. In LGCI, osteoclastogenesis and bone resorption are increased (11), leading to a perpetual feedback cycle (bone resorption ↔ inflammation).

Mesenchymal stem cells (MSC) are the common precursors for osteoblasts (bone forming cells) and adipocytes (30). However, inflammation may deregulate the MSC lineage commitment (11, 31). Even under normal homeostatic conditions, MSC slightly favors adipogenic differentiation. In LGCI, pro-inflammatory mediators in the presence of a high n-6 to n-3 ratio and reactive oxygen species (ROS), stimulate adipogenesis and suppress osteoblastogenesis, ensuring that MSC are committed to becoming adipocytes. In addition, high levels of IL-6, C-RP, and TNF-α are associated with lower muscle mass in older people (32). Since muscle and bone mass decline with age, and fat mass increases, it is possible that age-related MSC lineage commitment dysfunction occurring in LGCI and coinciding with obesity causes both osteopenia (loss of bone) and sarcopenia (loss of muscle). For the extensive review on the connection between bone, muscle, and fat, see Ilich et al. (31).

Recently, we investigated the effect of n-6 and n-3 PUFAs in different ratios on MSC proliferation and differentiation and found that the n-6 to n-3 ratio of 4:1 resulted in maximal osteoblastogenesis and minimal adipogenesis in a cell culture system (33). This ratio is higher than in the prehistoric humans (e.g. 1:1), and is feasible for modern humans with some dietary modifications. Figure 5 illustrates the loop effect of modern (A) and prehistoric (B) diet on adipogenesis, osteoblastogenesis, and osteoclastogenesis.
Proposed mechanism of action

The enzyme COX-2 has a higher affinity for n-6 than n-3 PUFA and the former will be metabolized at a higher rate (13, 14). However, higher presence of n-3, especially at the 1:1 ratio, would increase the odds of more n-3 being metabolized, which would lower the production of inflammatory eicosanoids. Some COX-2 inhibitors have been observed to decrease osteoblast differentiation and increase adipogenesis (34). This indicates that COX-2 and its lipid products may have a role in promoting MSC differentiation into adipocytes rather than osteoblasts, and that the ratio between n-6-derived COX-2 and n-3-derived COX-2 products may be important in MSC regulation.

Another proposed mechanism concerns the receptor activated nuclear factor kappa-B (RANK) signaling and osteoprotegerin (OPG). RANK binds to its ligand (RANKL), which is secreted by preosteoblasts, and activates osteoclastogenesis and bone resorption. Conversely, OPG, which is also produced by preosteoblasts, inhibits osteoclastogenesis by binding to RANKL, and thus promotes osteoblastogenesis. Upregulated pro-inflammatory cytokines, including TNF-α, IL-1, and IL-6 in obese state seem to mediate osteopenia and osteoporosis by regulating the RANKL/RANK/OPG pathway (31, 35).

CONCLUSION

Humans evolved on a diet low in saturated fat, carbohydrates, and sodium and much higher in calcium, vitamin C, fiber, and phytochemicals. Modern humans, however, consume high amounts of n-6 PUFA, about 10-15 higher than hominines. This elevates the ratio between n-6 to n-3 PUFA that stimulates pro-inflammatory processes and LGCI and promotes the pathogenesis of many chronic diseases, including obesity and osteoporosis. In contrast, n-3 PUFA and their derivatives, consumed by hominines in higher amounts, have been shown to promote anti-inflammatory processes and to have multiple beneficial health effects.

Obese individuals, who consume greater amounts of n-6 PUFA and exhibit higher circulating levels of pro-inflammatory cytokines (TNF-α, IL-6, C-RP), create the environment of a persistent LGCI, resulting in a perpetual feedback cycle. Furthermore, some cytokines (e.g. IL-6) stimulate osteoclastogenesis and

![Diagram of fatty acid metabolism and eicosanoids](diagram.png)
Figure 3 Hypothetical representation of acute versus low-grade chronic inflammation. The acute process is linear, since the inflammation is resolved, whereas the chronic process is a constant cycle in which the inflammation is persistent and unresolved. Cytokines are represented by colored shapes. Adapted from (11)

Figure 4 The increase in fat mass leads to overproduction of interleukin-6 (IL-6), interleukin-1 (IL-1), and tumor necrosis factor alpha (TNF-α). These cytokines increase bone resorption by stimulating the osteoclasts and inhibiting the osteoblasts.
are considered osteoresorptive. Both osteoblasts and adipocytes share MSC as their common precursor in the bone microenvironment. In conditions of LGCI, pro-inflammatory mediators in the presence of a high n-6 to n-3 ratio and reactive oxygen species stimulate adipogenesis and suppress osteoblastogenesis, shifting MSC differentiation toward adipocytes. The presented evidence shows that the increased synthesis of pro-inflammatory cytokines, mediated by the high n-6 to n-3 ratios, propagate obesity and osteoporosis by increasing or maintaining LGCI. In view of the recognized changes in dietary habits of modern humans compared to our ancestors, it would be prudent to decrease the consumption of n-6 fatty acids toward a more beneficial n-6 to n-3 PUFA ratio of 4:1 (as opposed to the current 15:1) in order to reduce obesity and improve bone health.

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Sažetak

Stalna kronična upala niskoga stupnja zbog suvremenog načina prehrane potiče pretilost i osteoporozu

Pojedina univerzalna svojstva prehrane hominina prije pojave poljodjelstva izrazito se razlikuju od suvremene prehrane. Prehrambene navike hominina vjerojatno su bile ograničene na plodove divljeg bilja i lovinu; više od 70 % moderne prehrane odnosi se na konzumaciju rafiniranih šećera, biljnih ulja, žitarica i mlječnih preradevina. Moderna je prehrana s višim unosom masti također dovela do nepovoljnijeg omjera omega-6 i omega-3 višestruko nezasićenih masnih kiselina (engl. krat. PUFA), koji pridonosi održavanju kronične upale niskoga stupnja, a time i nastanku mnogih kroničnih bolesti, uključujući pretilost i osteoporozu. U ovom se preglednom članku opisuju promjene uslijed modernog načina prehrane, s posebnim osvrtom na vrste i količine konzumirane masti. Također se objašnjavaju nedostatci moderne prehrane s obzirom na upalne procese te međusobna povezanost između pretilosti i upalnih procesa, koji su usto i poveznica između pretilosti i osteoporoze. U članku se iznose saznanja o tome da pretjerana konzumacija omega-6 masnih kiselina uz nedostatnu konzumaciju omega-3 masnih kiselina dovodi do kronične upale niskoga stupnja i povišenih vrijednosti reaktivnih kisikovih čestica (ROS), a time i do pomaka u mezenhimskih matičnih stanica (prekursora osteoblasta i adipocita) prema povećanoj adipogenezi i smanjenoj osteoblastogenezi. Uz povećanu sintezu upalnih citokina zbog pretilosti, moderna prehrana s nepovoljnim omjerom omega-6 i omega-3 kiselina u korist prvih nastavlja poticati pretilost i osteoporozu jer održava i pogoršava kroničnu upalu.

KLJUČNE RIJEČI: adipociti; citokini; ikosanoidi; mezenhimske matične stanice; omega-3 masne kiseline; omega-6 masne kiseline; osteoblasti; osteoklasti; višestruko nezasićene masne kiseline

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