Introduction

Obesity is the abnormal accumulation of adipose tissues. While the classic function of adipose tissue is to store fatty acids after food intake, and to release them during fasting state to keep energy status balanced [1], accumulating evidence indicates that adipose tissue is also actively involved in a variety of metabolic regulations. Obesity is closely related to blood pressure elevation, HDL decrease in blood, impaired glucose metabolism, and atherogenic dyslipidemia, which all together, are referred to as metabolic syndrome [2], leading to high susceptibility of cardiovascular disease and type 2 diabetes mellitus [3-4]. The rapidly increasing prevalence of metabolic syndrome was predicted to be one fourth of the populations in U.S. in the very near future [2].

Obesity is a chronic inflammatory disease [5], with macrophage infiltrating [6] and dysregulation of production of proinflammatory and anti-inflammatory cytokines [7] in adipose tissue. Toll like receptor 4 (TLR4) is a membrane spanning receptor which can induce the activation of nuclear factor-κB (NF-κB), a critical regulator of inflammatory responses [8], when binding to LPS ligand [9-10]. The NF-κB signaling pathway was found to be activated in insulin responsive tissues like liver, skeletal muscle and adipose tissue [11-14]. Recent studies demonstrated that saturated fatty acids activate TLR4-mediated NF-κB, and unsaturated fatty acids impede TLR4-mediated NF-κB activation [15-16]. Interestingly, It has been shown that the ratio of palmitic acid (16:0) to linoleic acid (18:2) is significantly greater in epididymal adipose tissue of obese mice (ob/ob) than that of normal non-obese mice [17], suggesting that fatty acids in adipose tissue may contribute to inflammation observed in obesity. Nevertheless, whether the same mechanism can be applied to other tissues besides adipose tissue,
Fatty acid profile in non-adipose tissues

contributing to inflammatory responses in obesity, still remains to be elucidated.

The objective of this study was to determine the fatty acid profile in non-adipose tissues from obese mice and compare it with the established role of fatty acids from adipose tissue. We report that the difference of saturated and unsaturated fatty acids composition in adipose tissue of obese mice compared with lean mice is opposite in non-adipose tissues, such as liver, spleen and pancreas, suggesting that saturated fatty acids per se may not contribute to inflammation in these non-adipose tissues.

Methods and methods

Animals

db/db obese mice [C57BLKS/J-leprdb/db] and lean mice [C57BLKS/J] were purchased from Jackson laboratory. The animals were fed with standard laboratory chow diet and used at 4-month old. Animal care and experiments were approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

Quantification of fatty acids with gas-chromatography/mass spectrometer (GC/MS)

Quantification of fatty acids was conducted as we previously described[18]. Briefly, epididymal adipose tissues obtained from db/db obese and lean mice were homogenized in chloroform (containing 100 μg BHT/ml) with a glass rod. Liver, spleen and pancreas tissues from obese and lean mice were homogenized in MBST/OG buffer with a douncer pestle and the lipids were extracted with Folch/BHT reagent [19] and lower phase of the extract was collected and dried under N₂. 50 μl Heptadecanoic acid (17:0) (5 mg/ml chloroform) was added to extract as an internal standard. Tissue total lipids were methyl esterified with BF3/Methanol (Supelco). The fatty acid methyl ester was analyzed using a gas chromatography system, Agilent 6890 GC G2579A system (Agilent) equipped with an OMEGAWAX 250 capillary column (Supelco) and a flame ionization detector. An Agilent 5973 mass selective detector was used to identify target peaks.

HEK-Blue cell culture and analysis of TLR4-mediated NF-κB Activation

HEK-Blue cells (InvivoGen) stably expressing TLR4, CD14, MD2, and a NF-κB reporter were cultured in a 96-well plate in complete DMEM (Invitrogen) containing high glucose 4.5 g/L, 10% Endolow FBS, 1% P/S, 1% glutamine, Nor-mocin and Selection until 90% confluency and treated with 200 μl 100 μM d-BSA bound saturated or unsaturated fatty acid solution for 24 h. Cells treated with 200 μl 1% d-BSA in PBS solution for 24 h were used as a control. Then, 100 μl of the culture supernatant was mixed with 100 μl HEK-Blue detection medium and incubated at 37 °C for 2 h. Activated TLR4 induces NF-κB reporter expression, catalyzing the HEK-Blue detection medium to blue. Absorption at 650 nm was measured to quantify the blue color.

Statistical analysis

Data are expressed as means ± SD. Data analysis was performed using two-sided Student’s t-test, and differences are considered significant at P < 0.05.

Results

Increased saturated fatty acid and reduced unsaturated fatty acid in adipose tissue of obese mice

Early study showed that the ratio of palmitic acid (16:0) to linoleic acid (18:2) is significantly greater in epididymal adipose tissue of obese mice (ob/ob) than that of normal non-obese mice [17]. To confirm the finding, we determined fatty acid profile in the adipose tissue of obese mice (db/db) and lean mice. As shown in Table 1, myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and α-linoleic acid (18:3) were identified from adipose tissue. These fatty acids comprise > 95% of total fatty acids. Compared to lean mice, obese mice had significant increases in saturated fatty acids, a 22% increase in palmitic acid and 55% increase in stearic acid, and a significantly decrease in unsaturated fatty acids, a 20% reduction in linoleic acid (P < 0.01, Figure 1).

Reduced saturated fatty acids and increased unsaturated fatty acids in non-adipose tissues of obese mice

We then asked whether there are similar changes on fatty acid profile in non-adipose
Fatty acid profile in non-adipose tissues

Table 1. Fatty acid composition in adipose tissue of obese mice and lean mice

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>C14:0</th>
<th>C16:0</th>
<th>C16:1</th>
<th>C18:0</th>
<th>C18:1</th>
<th>C18:2</th>
<th>C18:3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean mice fatty acid composition %</td>
<td>1.29</td>
<td>21.68</td>
<td>4.52</td>
<td>3.21</td>
<td>29.15</td>
<td>37.40</td>
<td>2.31</td>
</tr>
<tr>
<td>Obese mice fatty acid composition %</td>
<td>1.53</td>
<td>26.85</td>
<td>5.44</td>
<td>4.99</td>
<td>29.34</td>
<td>29.29</td>
<td>2.49</td>
</tr>
</tbody>
</table>

Saturated fatty acids promote TLR4-mediated NF-κB activation; in contrast, unsaturated fatty acids impede TLR4-mediated NF-κB activation.

NF-κB induces the expression of proinflammatory cytokines in human adipose tissue [14]. Using a luciferase assay, early studies demonstrated that saturated fatty acids induce TLR4-mediated NF-κB activation, and unsaturated fatty acids impede TLR4-mediated NF-κB activation [15-16]. Here we employed an alternative operating technique, HEK-Blue cell system, to elucidate the effect of fatty acid on TLR4-mediated NF-κB activation. The HEK-Blue cell stably expresses TLR4, CD14, MD2, and a NF-κB reporter. So it is a simple and convenient system to test TLR4-mediated inflammatory signaling. As shown in Figure 3, NF-κB activation was upregulated by stearic acid compared to BSA control (P < 0.01, Figure 3). In reverse, arachidonic acid attenuated TLR4-mediated NF-κB activation remarkably (P < 0.01, Figure 3).

Figure 1. Fatty acid composition in adipose tissue—obese mice versus lean mice. Mice epididymal adipose tissues were extracted with chloroform (containing 100 μg BHT/ml), followed by methylesterification of total fatty acids with BF3/Methanol. Analysis of fatty acids was performed using a gas chromatography system, and heptadecanoic acid (17:0) was used as an internal standard for data analysis. Adipose tissues of obese mice have increased saturated fatty acids and decreased unsaturated fatty acids. Dark bars are representative of obese mice, and light bars represent lean mice. n = 10. Data are expressed as means ± SD. **P < 0.01 versus lean mice.

tissues in obese mice versus lean mice. We quantified fatty acids in liver, spleen and pancreas in obese and lean mice. Unexpectedly, in liver of obese mice, the unsaturated fatty acids distribution was significantly increased compared with lean mice, 20% increase in oleic acid and linoleic acid, (P < 0.05, Figure 2A). In contrast, the saturated fatty acids, palmitic acid and stearic acid were reduced by 15% and 32%, respectively, in obese mice (P < 0.01, Figure 2B). Similar to the liver, the increase in unsaturated fatty acid and decrease in saturated fatty acid distribution was observed in the spleen in obese mice, a 70% increase in oleic acid and 50% increase in linoleic acid, and 32% and 60% decline of palmitic acid and stearic acid, respectively (P < 0.05, Figure 2B). Similar data were also obtained in pancreas: a 50% and 75% decrease in palmitic acid and stearic acid, and a 130% increase in oleic acid and 113% increase in linoleic acid in obese mice (P < 0.01, Figure 2C).
Palmitic acid seemed to enhance NF-kB activation as well but to a lesser extent compared to stearic acid. Oleic acid displayed inhibitory effect on TLR4-mediated NF-κB activation, but due to the relatively high value of standard deviations, the difference was not statistically significant (Figure 3).

Discussion

In this study, we compared the adipose tissue fatty acid profile between obese and lean mice. Our data confirm the early report that the ratio of palmitic acid (16:0) to linoleic acid (18:2) is significantly greater in epididymal adipose tissue of obese mice than that of normal non-obese mice [17]. As shown in Table 1 and Figure 1, there is a significant increase in stearic acid ratio in adipose tissue from obese mice compared with that from lean mice. Based on Figure 3, the stearic acid (18:0) is a more potent stimulator of TLR4 than palmitic acid (16:0). Thus, the stearic acid may be a major stimulator of inflammation among fatty acids in adipose tissue.

We then compared the non-adipose tissue fatty acid profile between obese and lean mice to determine whether the increase in saturated and decrease in unsaturated fatty acid presents in non-adipose tissue. Oppositely, we found that the non-adipose tissues, such as liver, spleen and pancreases, have significantly lower saturated and higher unsaturated fatty acid distribution in obese mice compared with that of lean mice. Based on Figure 3, the fatty acid profile of...
Fatty acid profile in non-adipose tissues

non-adipose tissue implies that the fatty acids from non-adipose tissue per se may play an inhibitory role in modulating TLR4/NF-κB signaling in obese mice. We should point out that other factors in non-adipose tissues also contribute to inflammatory response in non-adipose tissues.

Acknowledgements: This work was supported by grants to XAL from American Heart Association (0530241N), NIH (R01GM085231 and 3R01GM085231-02S1) and Children's Miracle Network.

Abbreviations: TLR4: Toll Like Receptor 4; NF-κB: Nuclear Factor-κB; BF3: Boron Trifluoride; DMEM: Dulbecco's Modified Eagle's Minimal Essential Medium; FBS: Fetal Bovine Serum; PBS: Phosphate Buffered Saline; d-BSA: Defatted Bovine Serum Albumin

Please address correspondence to: Xiang-An Li, PhD, MDS 401B, 725 Rose Street, Lexington, KY, 40536. Tel: 859-257-5113, Fax: 859-257-2120, E-mail: xli2@email.uky.edu

References