Trans Fatty Acid Intake Modulates the Expression of Uncoupling Proteins 2 and 3 (UCP2 & UCP3) mRNA in Children

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Abstract
Increased consumption of Trans Fats is associated with increased risk of Coronary Heart Disease. Uncoupling proteins (UCPs) are mitochondrial proteins that disperse the inter-membrane electrochemical potential as heat. We aimed to detect the relation of high Trans-fat intake in diet on the expression of UCP 2 & 3 m-RNA in children. A specific questionnaire to parents of sixty-eight children (4-15 years) was conducted. Accordingly, the subjects were sub-grouped into; High Trans-fat consuming group (37 subjects) and a Medium Trans-fat consuming one (31 subjects). Samples collected from Peripheral blood to analyze UCP 2 & 3 mRNA expression by Real Time Polymerase Chain Reaction (RT-PCR). Levels of UCP2 expression was reduced in children consuming High Trans-fat (2.5 ±0.7) in comparison with Medium Trans-fat consuming ones (1.5± 0.2) with (p<0.001). However, not much significance was showed in UCP3 expression with values (2.1±0.5) in the High consuming group and (1.9±0.2) in Medium consuming group with (p=0.08). In Delta relationship the diet-induced changes in UCP2 (r=0.66, P=0.002) and UCP3 (r=0.61, P=0.06) mRNA expression was negatively correlated with percentage of Trans-fat in diet. The correlation of UCP 2 & 3 mRNA expression and high Trans-fat intake suggests a mechanism by which high Trans-fat diet plays a role in childhood obesity.

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Highlights
- Children in Egypt showed high consumption of Trans-fat.
- Levels of UCP2 and UCP3 mRNA expression are negatively correlated with the amount of Trans-fat intake.

Introduction
Trans fatty acids (TFA) are generally geometrical isomers of unsaturated fatty acids and have at minimum one non-conjugated carbon-carbon double bond in the form of trans-configuration. Dietary TFA could be of artificial or organic sources. The artificial TFA are generated by partial hydrogenation of unsaturated vegetable oils. The major sources of artificial TFA in food are margarines, deeply fried fast foods, commercially baked products and packaged snack foods.

Consuming high amounts of TFAs (>1% of total daily energy intake) is associated with high risk of Coronary Heart Disease (CHD). That could be linked to increase low-density lipoprotein cholesterol (LDL), decrease high-density lipoprotein cholesterol (HDL), release of pro-inflammatory cytokines and endothelial dysfunction. Moreover, high TFA has been identified as a major risk factor for obesity. Lesions in the big arteries can begin to appear in childhood and are majorly linked to dyslipidemia. The negative health effects of TFA were attributed mainly to TFA coming from artificial origin and some studies have suggested that natural TFA might have beneficial effects.

Non-communicable disease (NCDs) is accused of about 68% of deaths in the Eastern Mediterranean Region (EMR). Elevated numbers of children (6.9% of children under five years) higher than the global average of (6.2%) showed overweight. In most EMR countries, more than 50% of the adolescents are overweight or obese. In 2016 The total mortalities of cardiovascular diseases (CVDs) in the EMR was around 1.3 million, representing 31.9% of total mortality rate.

Uncoupling proteins (UCPs) biochemically are proteins disperse the mitochondrial inter-membrane electrochemical potential as heat. UCPs are classified UCP-1, 2 and 3. UCP-1 is overexpressed when exposing to cold and overeating, and its half-life ranges from hours to days. The second isoform, UCP-2, has shown to mediate cyto-protection, immune cell modulation and enhance glucose sensitivity in tissues as the brain and the pancreas, having a half-life of only 1h. Lastly, UCP-3 is markedly translated in skeletal tissue, with a half-life of 1 to 4h. UCP3 could be of high thermo-genic importance under specific situations and it is an attractive drug target for the management...
of obesity. A major function of UCP 2 & 3 is to attenuate mitochondrial production of free radicals in the mitochondria, in cells fighting against oxidative damage.\textsuperscript{15}

Activation of proton transport of UCP2 by Reactive oxygen species (ROS) reduces insulin production in pancreatic β cells. This may be pathologically negative effect of the preservative mechanism that restricts production of ROS and damage of pancreatic islet during high fat feeding or hyperglycemia and causes type II diabetes.\textsuperscript{16} This action could be considered a physiological mechanism to control the use of fatty acids and glucose as sources of energy.\textsuperscript{17}

\textbf{Subjects and Methods}

\textbf{Subjects}

The subjects comprised 68 child (31 males and 37 females, mean age 9 ± 6yr) visiting Zagazig University hospital clinics - Egypt. Approval for the study was obtained from the Institutional Review Board (IRB). Dietary Trans-fat intake was assessed using a 162-item semi quantitative food frequency questionnaire (SFFQ). The questionnaire was designed to evaluate socio-demographic data, nutritional habits and the frequency of consuming selected foods. There were 5 options for respondents to choose from, ranging from never to daily with determination of the sizes. We asked their parents about the type of fat ordinarily used for baking and frying food and at the table. All procedures performed in our study were in accordance with the ethical standards of the institution and an informed consent approval from the parents. Composition values for dietary fats and other nutrients were obtained from National Nutrition Institute Food Composition Tables for Egypt\textsuperscript{18} with the help of other reports to determine Trans-fat contents.\textsuperscript{19,20, 21}

After analysis of the food frequency questionnaire data, the amount of Trans fat in diet were estimated and accordingly the subjects were sub-grouped into: High Trans-fat consuming group included 37 individuals with a mean ± SD (2.2 ± 0.36) g per day, which represented 11.2% of dietary fat, Medium Trans-fat consuming group comprised 31 subjects with a mean ± SD (1.2 ± 0.22) g per day, which represented 6.1% of dietary fat which comprised 31 children.

\textbf{Collection of Blood Samples}

Three ml of whole blood was collected from each participant under complete aseptic condition in sterile EDTA (solute form) containing tubes and kept frozen at -20°C till analysis.

\textbf{RNA Extraction}

The RNA extraction was done using The IQeasy TM plus Blood RNA Extraction Mini Kit purchased from iNtRON Biotechnology. Evaluation of purity and concentration of each RNA sample was done by determination of the A\textsubscript{260}/A\textsubscript{280} ratio using Milton Roy Spectronic 3000 Array.

\textbf{Reverse Transcription}

The cDNA was prepared by reverse transcription of total RNA template using Maxime RT PreMix Kit purchased from iNtRON Biotechnology.

\textbf{Table 1: Primers for PCR}

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sense Primer (5’-3’)</th>
<th>Antisense Primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP-2</td>
<td>GACCTATGACCTCATCAAGG</td>
<td>ATAGGTGACGAACATCACCAG</td>
</tr>
<tr>
<td>UCP-3</td>
<td>ATGGACGCCTACAGAACCAT</td>
<td>TACGAACATCACCACGTTCC</td>
</tr>
<tr>
<td>β-actin</td>
<td>AGAGCTACGAGCTGCCTGAC</td>
<td>AGCACTGTGTTGGCGGTACAG</td>
</tr>
</tbody>
</table>

\textbf{Real-Time qPCR}

2\mu l of first-strand cDNA was used for real time PCR (RT-PCR) in 20\mu l reactions containing 10 ul of qPCR Green Master, 1ul of each primer and 4ul PCR grade water. To avoid co-amplification of the genomic DNA, that may cause contamination of the RNA preparation, we designed All primer combinations to span at least one intron. The primers used for amplifying cDNA fragments are shown in Table 1. PCR conditions were as follows for 40 cycles: 1 min at 94°C, 45 sec at 57°C and 2 min at 72°C proceeded by initial denaturation at 95 °C for 10 min to fully activate the chemically modified PCR DNA polymerase and were quantified by Agilent
Mx3005P Real-Time PCR Systems. Levels of mRNA were cleared up as the ratio of signal intensity for the target gene relative to β-actin. All the determinations were performed in duplicate.

**Statistical Analysis**

Statistical analysis was conducted using the statistical package for Social Science (SPSS) version 23. All results are presented as the means ± SD (standard deviation) and p < 0.05 was considered statistically significant.

**Results**

For both genders, the age ranged from 4 to 15 years. More than half of the parents felt they had sufficient money. Thirty eight percent of the sample was overweight (BMI > 25 kg/m²). Daily food consumption was similar for both genders Table 2.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Females</th>
<th></th>
<th>Males</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td><strong>Age Group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 – &lt;10</td>
<td>15</td>
<td>22%</td>
<td>18</td>
<td>27%</td>
</tr>
<tr>
<td>10 – 15</td>
<td>22</td>
<td>32%</td>
<td>13</td>
<td>19%</td>
</tr>
<tr>
<td><strong>Economic situation (income)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mostly sufficient</td>
<td>22</td>
<td>32.50%</td>
<td>20</td>
<td>29.50%</td>
</tr>
<tr>
<td>Mostly insufficient</td>
<td>14</td>
<td>21%</td>
<td>11</td>
<td>16.50%</td>
</tr>
<tr>
<td>More than enough</td>
<td>1</td>
<td>0.50%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Below normal (&lt;18.5 kg/m²)</td>
<td>10</td>
<td>15%</td>
<td>8</td>
<td>12.50%</td>
</tr>
<tr>
<td>Normal (18.5–25 kg/m²)</td>
<td>12</td>
<td>17%</td>
<td>12</td>
<td>17%</td>
</tr>
<tr>
<td>Overweight (&gt;25 kg/m²)</td>
<td>15</td>
<td>22%</td>
<td>11</td>
<td>16.50%</td>
</tr>
<tr>
<td><strong>Food frequency questionnaire (FFQ)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweets</td>
<td>36</td>
<td>53%</td>
<td>30</td>
<td>44.50%</td>
</tr>
<tr>
<td>Cake, cookies</td>
<td>33</td>
<td>48.50%</td>
<td>29</td>
<td>43%</td>
</tr>
<tr>
<td>Snacks</td>
<td>34</td>
<td>50%</td>
<td>20</td>
<td>29%</td>
</tr>
<tr>
<td>Fresh fruits</td>
<td>34</td>
<td>50%</td>
<td>20</td>
<td>29%</td>
</tr>
<tr>
<td>Salad, raw vegetables</td>
<td>30</td>
<td>44.50%</td>
<td>18</td>
<td>26.50%</td>
</tr>
<tr>
<td>Cooked vegetables</td>
<td>29</td>
<td>43%</td>
<td>17</td>
<td>25%</td>
</tr>
<tr>
<td>Fast food, canned food</td>
<td>31</td>
<td>46%</td>
<td>30</td>
<td>44.50%</td>
</tr>
<tr>
<td>Lemonade, soft drinks</td>
<td>20</td>
<td>29%</td>
<td>13</td>
<td>19%</td>
</tr>
<tr>
<td>Meat, sausages</td>
<td>29</td>
<td>43%</td>
<td>27</td>
<td>40%</td>
</tr>
<tr>
<td>Fish, sea food</td>
<td>20</td>
<td>29%</td>
<td>20</td>
<td>29%</td>
</tr>
<tr>
<td>Milk and milk products</td>
<td>37</td>
<td>54%</td>
<td>30</td>
<td>44.50%</td>
</tr>
<tr>
<td>Cereals and their products</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Saturated fat intake was correlated with both monounsaturated fat ($r = 0.73$) and trans-fat ($r = 0.5$) intake, but not with polyunsaturated fat ($r = 0.01$). Monounsaturated fat intake was correlated with both trans-fat ($r = 0.57$) and polyunsaturated fat ($r = 0.5$) intake. Polyunsaturated fat intake was correlated with trans-fat ($r = 0.57$) intake only. Common daily dietary sources could be the cause of this high correlation between monounsaturated fatty acids and saturated fatty acids.

On average, Total fat consumption was about 18.5 ± 0.7% of the total calories. Mean reported
energy with trans-fat contributes to ≥ 2.3± 0.9% of the total calories and fat intake were similar for males and females, although the range of intake was more among the females.

Table 3: mRNA expression in relation to Trans Fat Consumption

<table>
<thead>
<tr>
<th></th>
<th>High Trans fat consuming</th>
<th>Medium Trans fat consuming</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCP2 mRNA expression</td>
<td>2.5 ± 0.7</td>
<td>1.5 ± 0.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>UCP3 mRNA expression</td>
<td>2.1 ± 0.5</td>
<td>1.9 ± 0.2</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Effect of the Trans-Fat Content of Diet on UCP2 and UCP3 mRNA Expression

UCP2 mRNA expression was lower in the High Trans-fat consuming group compared to Medium Trans-fat consuming ones (2.5 ± 0.7 vs. 1.5 ± 0.2, p < 0.001). However, UCP3 mRNA expression was not significantly different between both groups (2.1 ± 0.5 vs. 1.9 ± 0.2, P=0.08) Table 3.

In relationship between Delta (= mRNA expression on Medium Trans-fat consuming group minus High Trans-fat consuming one) we found that the diet-induced changes (delta) in mRNA expression of UCP2 (r = 0.66, P = 0.002) and UCP3 (r = 0.61, P = 0.06) mRNA expression (ratio with β-actin) were negatively correlated with percentage of Trans fat in diet.

Discussion

The major sources of trans fats are meat or dairy products if we considered natural TFA. On the other hand, margarines, deeply fried fast foods, commercially baked products, packaged snack foods and other prepared foods are rich with artificial TFA. Increased consumption of trans fats has been associated with increased risk of Coronary Heart Disease (CHD) morbidity and mortality. Moreover, high TFA has been identified as a risk factor for obesity.

Uncoupling proteins types 2 and 3 are suggested to be involved in energy metabolism and obesity development. UCP2 is expressed in most of human tissues. Initially, a lot of studies had reported the role of UCP2 in the regulation of energy homeostasis and metabolism. Moreover, the UCP2 expression has been linked to obesity, metabolic syndrome and basal metabolic rate alterations. Vidal-Puig, A. J. concluded that; UCP2 and UCP3 are not thermogenic to UCP1 degree. Moreover, UCP2 and UCP3 genes expression was detected to be as 1000-fold less than UCP1 expression. Activation of all UCP types requires fatty acids. Schrauwen P and Hesselink MK suggested that UCP3 transport fatty acids out of mitochondria thus protecting the mitochondria from the toxic effects of fatty acid peroxidation and trans fats.

Uncoupling proteins (UCPs) are mitochondrial proteins, which act by increasing the permeability of the inner mitochondrial membrane to protons returning them back into the mitochondrial matrix without energy production from the Electron Transport Chain. There are three isoforms discovered: UCP-1, 2 and 3. UCP-1 expression is increased following cold exposure and overeating. Previous study had investigated the relation between UCP2 and UCP3 expression and fasting. Based on the central role of the UCP2 and UCP3 in metabolism, in the present study we examined the effect of the Trans-fat food consumption on the expression of UCP2 and UCP3 mRNA in children.

The levels of UCP2 mRNA expression was lower in the High Trans-fat consuming children (2.5 ± 0.7) compared to Medium Trans-fat consuming ones (1.5 ± 0.2) with (p = 0.001). However, UCP3 mRNA expression levels didn’t show that much significance as the values were (2.1 ± 0.5) in the High Trans-fat consuming group. While showed (1.9 ± 0.2) Medium Trans-fat consuming one with p value (0.08). In relationship between Delta we found that the diet-induced changes in mRNA expression of UCP2 (r = 0.66, P = 0.002) and UCP3 (r = 0.61, P = 0.06) mRNA expression (ratio with β-actin) were negatively correlated with percentage of Trans fat in diet.
Rodríguez E et al showed similar findings and reported that Trans fatty acids inhibited uncoupling protein (UCP) 1 induction by norepinephrine (NE), decreased the leptin mRNA levels and caused defective brown adipose tissue (BAT) thermogenesis. Machado RM et al research results showed the role of trans fatty acid consumption in the development of the key features of metabolic syndrome. Jeyakumar SM et al concluded that the chronic consumption of a TFA-rich diet significantly impaired insulin sensitivity, which is a main characteristic for metabolic syndrome. Zhao X et al research results support our findings as they concluded that; consumption of high trans-fatty acids diet induces higher rates of obesity and insulin resistance.

In the same line of our data, Siagian CM results showed that high TFA intake without concurrent increase in calories caused an increase in intra-abdominal fat deposit and defective insulin sensitivity. In cooperation with Tjahjono DK, Siagian CM has emphasized his results in another study. Also, in agreement with our results, West DB et al reported that administration of conjugated linoleic acids (CLA) down regulates UCP2 expression. However, other studies have observed that a high fat diet influence UCP3 mRNA expression especially in skeletal muscle. Conversely, substitution with a low-fat diet was found to reduce UCP2 expression and increase ROS production. This could be attributed to correlating with total fat in diet including saturated, monounsaturated and polyunsaturated fats and not only with trans-fat consumption. And the effects on metabolism and gene expression depend on the type and source of lipids present in the diet and could be attributed to energy expenditure and adipose tissue metabolism, such as lipoprotein lipase, lipogenesis, and lipolysis activities.

Putting these findings together, indicate that the increased levels of trans fat consumption in diet affects the levels of UCP2 and UCP3 mRNA expression which in turn affects their levels. These effects are expected to have direct relation with obesity and its comorbidities. Our findings should have implications for additional clinical and laboratory research.

Conclusion
The levels of UCP2 and UCP3 mRNA expressions are reduced by high Trans-fat content in diet. This down regulation is more significant on UCP2. This gives a possible mechanism by which Trans-fat leads to obesity and metabolic syndrome in children.

Recommendations
Presenting the trans-fat content in the food composition tables for Egypt is required. And further studies with higher subject number with more demographic correlation is recommended.

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Conflict of Interest
The authors declare that they have no competing interests.

References


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