Circulating Plasma Free Fatty Acids, Insulin Resistance and Metabolic Markers in Obese Women

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Elevation of free fatty acids (FFAs) in serum is an important risk factor for metabolic changes. Conversely, the relationship between obesity and metabolic abnormalities, and FFAs is not yet completely understood. Thus, we aimed in this study to explore the relationship and the association between insulin resistance (IR), metabolic markers and the variation in plasma FFAs among the obese women. This study included fifty obese women aged 25–35 years and has insulin resistance (IR) in addition to fifty age-matched healthy normal weight women served as control group. Blood was withdrawn after twelve hours fasting; fasting blood glucose, lipids and plasma insulin were estimated; IR was assessed via the Homeostasis Model Assessment-Insulin Resistance (HOMA-IR). Fatty acids in plasma were measured by HPLC using UV detector that was set at 200 nm. Indeed, anthropometric measurements was performed. Lipid profile, fasting blood sugar, insulin resistance, oleic acids (OA), linoleic acid (LA), arachidonic acid (AA) and anthropometric measurements were significantly increased in IR women compared to control. Whereas, the mean value levels of alpha-linolenic acid (ALA) was significantly decreased in IR women compare to controls. Lower plasma levels of ALA and higher levels of AA, OA, LA were significantly associated with risk of IR and metabolic disorder markers in obese women. These results might explain the positive benefits of foods rich with poly unsaturated fatty acids (PUFA). Obesity and IR may be associated with the alterations in composition of the circulating fatty acid. These findings underscore the potential role of PUFA in the metabolic syndrome pathogenesis.

Keywords: Fatty acids; obese women; insulin resistance; metabolic markers.

Obesity is mainly in alliance with the risk of numerous diseases as nonalcoholic fatty liver, cardiovascular disease (CVD) and diabetes mellitus. When the nutrient intake exceeds the body needs, tissues such as adipose and skeletal and also other body organs like liver become saturated with lipids and resulting in an elevation of lipid export leading to liberation of huge amount of FFAs¹. Previous epidemiologic studies indicated that individuals with higher levels of plasma FFAs were at increased risk for type 2 diabetes (T2D)². Free fatty acids (FFAs) are an imperative energy resource human body, and attached to nuclear peroxisomal proliferated-activated receptors (PPARs) interposing genes expression implicated in the metabolism of both lipids and glucose³,⁴. AA, the omega – 6 fatty acid is found in the cell membrane phospholipids, and
the originator of a huge bioactive compounds family called eicosanoids, that are generated via its oxygenation. The liberation of AA from the cell membrane is depending on several enzymes. Additionally, elevation of FFAs levels is linked to insulin resistance through the reduction of glucose transporters and glycogen synthesis. It was found that plasma FFA levels are elevated in obese patients and it was hypothesized that increasing of FFA levels is an important mark of obesity-associated metabolic syndrome. In addition, obesity is associated with elevation of free radicals and oxidative stress that produced as normal end products of the cellular metabolism and also during inflammation process by phagocytosis. In adipose tissue insulin resistance leads to increased lipolysis and subsequently to increase in the liberation of free FAs, which is the chief source of triglycerides stored in the liver.

Consequently, we aimed in this study to give a clear picture about the relationship between insulin resistance and plasma fatty acid in obese women and assess its associations with metabolic markers.

**Subjects and Methods**

**Subjects**

This study involved 100 women (unrelated); 50 age-matched healthy women & 50 obese women with IR. Their age was among 21 and 36 years. These cases were indicated from diverse centers to the National Research Centre obesity clinic. The treatise has been authorized by the Ethical Committee of NRC, Egypt (number: 16361), in agreement with the World Medical Association’s Declaration of Helsinki.

**METHODS**

**Clinical and biochemical parameters**

BMI (Body mass index) was calculated as weight in kilograms divided by height in meters square (kg/m2). MUAC (Mid upper arm circumference) was measured by a resilient tape at the midway between acromial process on the upper right arm with the elbow flexed 90° and the olecranon. Hip circumference (HC) and Waist circumference (WC) were measured in cm. Waist-to-hip ratio (WHR) was calculated. Fat mass was measured by Tanita Body Composition Analyzer (SC-330).

After 12 hours fasting, blood was collected from all patients, and serum was separated. Blood glucose (fasting) was assessed immediately by enzymatic colorimetric method Centronic, Germany. Insulin level was assessed by ELISA. Whereas, insulin resistance (HOMA-IR) was calculated from the formula: Fasting plasma glucose (mmol/l) period serum insulin level(mIU/l) /405. High HOMA-IR values referred to high insulin resistance, whereas Low HOMA-IR values indicate high insulin sensitivity as described previously.

Aspartate amino transferase (AST) and alanine amino transferase (ALT) in serum were assessed using commercial kit from BioMed Diagnostics according to the method described by.

Serum triglycerides (TG) and serum total cholesterol (TC) were determined by enzymatic colorimetric method. Additionally, high-density lipoprotein cholesterol (HDL-C) was estimated. Dependent low-density lipoprotein cholesterol (LDL-C) was calculated from the equation mentioned before as follows:

\[
LDL - C = TC - (HDL - C + TG/5)
\]

**Estimation of fatty acids using HPLC**

Fractions of fatty acids were assessed using HPLC, Agilent technologies 1100, equipped with a quaternary pump (model G131A) as described previously.

Fatty acids HPLC standards grade (LA, ALA, OA, AA, DHA) were purchased from Sigma Chemical (Munich, Germany). Acetonitrile, methanol, ethanol, N-hexane, 2-propanol and other laboratory chemicals in this study were HPLC grade. Ultra-pure water was used for all experimental work and analysis.

**Sample preparation**

Plasma was homogenized in a solution consists of 2 % acetic acid: ethyl ether mixture (2:1) v/v. This solution was centrifuged at 3000 rpm using cooling centrifuge; the organic layer was evaporated under nitrogen gas until complete dryness. The resultant residue dissolved in acetonitrile (400 il) and filtered using hydrophilic PVDF 0.45 i m before injection.
HPLC condition

The technique was done by RP(reversed phase) HPLC column (260 X 4.6, particle size 5ìl) and the used mobile phase was consisted of 70 % acetonitrile by isocratic elution by flow rate 1 ml/ min and ;UV detector was at 200 nm. Sequential dilutions of each standard were injected and their corresponding peak zones were specified. The mean values of each fatty acid in all samples were calculated from the linear standard curve.

Statistical analysis

We performed the statistical analyses using SPSS16.0 for Windows (SPSS Inc). Two-tailed P<0.05 was considered statistically significant.

RESULTS

Table 1 displayed significant differences in anthropometric parameters between IR cases and controls. Obese IR women had significantly higher levels of BMI, body fat %, MUAC and WC than controls (p<.05). In addition, no significant changes were observed in fasting blood sugar, lipid profile, and liver functions between the two studied groups; however insulin and insulin resistance were significantly augmented in obese women compared to control (table 2, 3).

Table 4 appeared significant changes in fatty acids fractionation between obese women and control. Thus, the mean value level of OA,LA, and

<table>
<thead>
<tr>
<th>Table 1. Anthropometric measurements in studied groups</th>
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<tr>
<td>Variables</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Body mass index (BMI)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Body fat %</td>
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<tr>
<td></td>
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<tr>
<td>Mid upper arm circumference (MUAC)</td>
</tr>
<tr>
<td></td>
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<tr>
<td>WC</td>
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<td></td>
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<tr>
<td>WHR</td>
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</tbody>
</table>

All data are expressed as mean± SD
P: significant difference (<0.05) in insulin resistance (IR) group compared to control
P: High significant difference (<0.001) in insulin resistance (IR) group compared to control

<table>
<thead>
<tr>
<th>Table 2. Fasting blood sugar, insulin resistance and insulin in studied groups</th>
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<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Insulin ( IU/ml )</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>HOMA</td>
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P: significant difference (<0.05) in insulin resistance (IR) group compared to control
P: High significant difference (<0.001) in insulin resistance (IR) group compared to control
AA was significantly increased along with a significant reduction in ALA in obese group in comparison to control.

**DISCUSSION**

Obesity causes numerous metabolic dysregulations including alteration of lipid profile (cholesterol and triglycerides), besides glucose homeostasis including alteration of insulin and its resistance in addition to deterioration of pro and anti-inflammatory status. Owing to the hyperlipolytic properties of the visceral adiposity, surplus visceral fat liberates huge quantity of fatty acids; thus, inflow of fatty acids from visceral adipose tissues to the liver through the portal vein is augmented. Furthermore, Nielsen et al. elucidated that fatty acid liberation from visceral fat into hepatocytes influenced as visceral fat mass augmented. This leads to elevated fatty acid in hepatocytes. Accordingly, stimulating synthesis and secretion of TG in the liver through its integration into TG-rich lipoproteins like very low-density lipoproteins (VLDLs) circulating.

### Table 3. Liver functions and lipid profile in studied groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>Controls</td>
<td>15.38 ± 8.50</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>19.23 ± 18.07</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>Controls</td>
<td>20.22 ± 5.433</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>22.45 ± 13.50</td>
<td></td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>Controls</td>
<td>197.12 ± 37.38</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>195.60 ± 38.43</td>
<td></td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>Controls</td>
<td>98.86 ± 49.29</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>101.60 ± 40.88</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>Controls</td>
<td>47.84 ± 11.25</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>50.45 ± 13.54</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>Controls</td>
<td>128.58 ± 43.45</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>125.91 ± 43.376</td>
<td></td>
</tr>
</tbody>
</table>

All data are expressed as mean± SD

P: significant difference (<0.05) in insulin resistance (IR) group compared to control

P: High significant difference (<0.001) in insulin resistance (IR) group compared to control

### Table 4. Plasma fatty acids (µg/ml) in studied groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid (OA) µg/ml</td>
<td>Controls</td>
<td>4.53 ± 3.31</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>6.56 ± 3.50</td>
<td></td>
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<tr>
<td>Linoleic acid (LA)</td>
<td>Controls</td>
<td>6.13 ± 5.19</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>10.34 ± 4.14</td>
<td></td>
</tr>
<tr>
<td>Archidonic acid (AA)</td>
<td>Controls</td>
<td>7.12 ± 4.69</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>11.30 ± 4.79</td>
<td></td>
</tr>
<tr>
<td>alpha-linolenic acid (ALA)</td>
<td>Controls</td>
<td>4.54 ± 0.27</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>IR</td>
<td>2.41 ± 0.38</td>
<td></td>
</tr>
</tbody>
</table>

All data are expressed as mean± SD

P: significant difference (<0.05) in insulin resistance (IR) group compared to control

P: High significant difference (<0.001) in insulin resistance (IR) group compared to control
TG is augmented due to cumulating visceral fat. Furthermore, both the concentrations of the systemic circulating fatty acids and fatty acids in the portal vein levels observed positive and significant correlations with visceral adipose tissues. In this work, the mean value levels of omega-3 fatty acids were significantly decreased in obese women compared to control; whereas the mean value levels of omega6 & omega9 were significantly elevated in obese.

The elevation of omega 6 and 9 fatty acids and also the reduction of omega-3 in obese women in this study are linked to the elevation of insulin resistance as appeared in tables 2 and 4.

The composition of fatty acids could clarify a phenomena including the relationship between insulin and its receptors. It was indicated that, the cell membrane fatty acids composition of insulin target tissues, as skeletal muscle & liver, is an important factor that affects each of insulin production and its vital actions. Consequently, membranes enrich in omega-3 fatty acids like ALA have a tendency to bind more insulin than membrane enrich in omega-6 and 9 fatty acids. Elevation of free fatty acids like unsaturated and omega6 fatty acids results in increase of the fatty acyl-CoA (FACyl CoA) and diacylglycerol (DAG) concentrations, resulting in initiation and activation of protein kinase C isoform (PKC-α) which leads to elevation of insulin receptor substrate-1 (IRS-1) serine phosphorylation. Sequentially, a reduction of IRS-1 tyrosine phosphorylation & IRS-1 related phosphatidylinositol 3-kinase (PI3-K) activity causea reduction of insulin-stimulating glucose transport action18.

Contrarily, ALA improved insulin sensitivity via rising the responsibility of glucose transporter -4 (GLUT-4), that leads to a development of glucose-6-phosphate19. Indeed, Kato et al.,20 stated that GLUT-4 in ALA treated mice was better by 250% when compared to that in control group.

Concomitantly, Hussein et al.,21 indicated that flaxseed oil (a plant source of omega-3 fatty acids) has a positive impact on reducing insulin resistance in diabetic animals via scavenging properties of free radicals & increasing antioxidant enzymes. This impact may be due to the up regulation gene expression of antioxidants enzymes and down regulation gene linked with the establishment of free radicals22.

The composition of fatty acid (FA) in serum lipid esters is a mirror to particular extent the dietary composition of FA during the last 6 to 8 weeks. The serum FA pattern is also dependent on the metabolism of FA and their endogenous synthesis. Also depends on intrauterine & prenatal programming and genetic variation. Low levels of linoleic acid (18:2, n-6) & high levels of palmitic acid (16:0) in plasma are characteristic for individuals with metabolic syndrome and insulin resistance24.

Arachidonic acid (AA) acts as a powerful negative modulator of glucose uptake25 and researches have elucidated elevated serum levels of arachidonic acid in diabetic subjects in comparison with normal controls26. Thus, the data have been in agreement with researches that have shown a positive relationship between insulin resistance and AA20,21.

CONCLUSIONS

Obesity and IR may be associated with the alterations in composition of the circulating fatty acid. The current study appeared the association of omega6 and 9 fatty acids with insulin resistance and hyperlipidemia. Additionally, these findings underscore the potential role of UFAs in the MS pathogenesis.

ACKNOWLEDGMENTS

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Conflict of interest

All authors declared that they have no conflict of interest.

REFERENCES

8. Reitman S, Frankel S. A colorimetric method for
the determination of serum glutamic oxalacetic
9. Friedewald WT, Levy RI, Fredrickson DS.
Estimation of the concentration of low-density
lipoprotein cholesterol in plasma, without use of
the preparative ultracentrifuge. Clin. Chem., 18,
10. Hussein J, El-Naggar ME, Latif YA, Medhat
D, El Bana M, Refaat E, Morsy S. Solvent-free
and one-pot synthesis of silver and zinc oxide
nanoparticles: activity toward cell membrane
component and insulin signaling pathway in
experimental diabetes. Colloids Surfaces B
11. Hussein JS, El-Khayat Z, Morsy S, Oraby F,
Singer GG. The effect of fish oil on oxidant/
antioxidant status in diabetic rats through
the reduction of arachidonic acid in the cell
12. Hussein J, Attia MF, El Bana M, El-Daly SM,
Mohamed N, El-Khayat Z, El-Naggar ME.
Solid state synthesis of docosahexaenoic acid-
loaded zinc oxide nanoparticles as a potential
antidiabetic agent in rats. Int. J. Biol. Macromol.,
13. Després J-P. Health consequences of visceral
14. Girard J, Lafontan M. Impact of visceral adipose
tissue on liver metabolism and insulin resistance.
Part II: Visceral adipose tissue production and
liver metabolism. Diabetes Metab., 34, 439–45
(2008).
15. Lafontan M, Girard J. Impact of visceral adipose
tissue on liver metabolism: Part I: Heterogeneity
of adipose tissue and functional properties of
visceral adipose tissue. Diabetes Metab., 34,
16. Nielsen S, Guo Z, Johnson CM, Hensrud DD,
17. Jensen MD. Is visceral fat involved in the
19. Dey D, Bhattacharya A, Roy S, Bhattacharya
S. Fatty acid represses insulin receptor gene
expression by impairing HMGA1 through
T, Tanigawa K. Effect of alpha-linolenic acid on
blood glucose, insulin and GLUT4 protein
content of type 2 diabetic mice. J. Heal. Sci., 46,
E, Khateeb S. Insulin resistance and oxidative
stress in diabetic rats treated with flaxseed oil. J
22. Harding AH, Day NE, Khaw KT, Bingham SA,
Luben RN, Welsh A, Wareham NJ. Habitual
fish consumption and glycated haemoglobin:
23. Vessby B, Gustafsson I, Tengblad S, Boberg M,
Andersson A. Desaturation and elongation of
24. Vessby B. Dietary fat, fatty acid composition in
plasma and the metabolic syndrome. Curr. Opin.
25. Tebbey PW, McGowan KM, Stephens JM,
Buttte TK, Pekala PH. Arachidonic acid
down-regulates the insulin-dependent glucose
transporter gene (GLUT4) in 3T3-L1 adipocytes by
inhibiting transcription and enhancing mRNA
26. Simopoulos AP. Omega 6/Omega 3 Fatty Acid
Ratio and Trans Fatty Acids in Non Insulin