Biomedical & Pharmacology Journal, December 2020. Vol. **13**(4), p. 1595-1600

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

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https://dx.doi.org/10.13005/bpj/2034

(Received: 15 September 2020; accepted: 14 December 2020)

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 Blood was withdrawn after twelve hours fasting; fasting blood glucose, lipids and plasma insulin were estimated;IR was assessed via the Homeostasis Model Assessment-Insulin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 FFAs[1] . 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) interposinggenes expression implicated in the metabolism of both lipids and glucose[3,4]. 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[5].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 is involved 100 women(unrelated); 50age-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^{o}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 [6]. 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(mU/l) /405. High HOMA-IR values referred to high insulin resistance, whereas Low HOMA-IR values indicate high insulin sensitivity as described previously[7] .

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[8] .

Serum triglycerides (TG) and serum total cholesterol (TC) were determined by enzymatic colorimetric method. Additionally, high-density lipoprotein cholesterol (HDL-C) was estimated. Dependently low-density lipoprotein cholesterol (LDL-C) was calculated from the equation mentioned before[9]as follow:

LDL – C = TC – (HDL- C + TG/5)

Estimation of fatty acids using HPLC

Fractions of fatty acids were assessed sing HPLC, Agilent technologies 1100, equipped with a quaternary pump (model G131A) as described previously[10,11].

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[12].

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 ìl)and filtered using hydrophilic PVDF 0.45 ì m before injection.

HPLC condition

The technique was done by RP(reversed phase) HPLC column (260 X 4.6, particle size 5il) 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 1. Anthropometric measurements in studied groups

Variables	Group	Mean ± SD	P value
Age	Controls	33.67±10.735	0.121
	IR	36.24 ± 9.595	
Body mass index (BMI)	Controls	23.05 ± 4.65	0.05
	IR	28.01± 6.63	
Body fat %	Controls	23.71 ± 8.61	0.001
	IR	35.52 ±12.93	
Mid upper arm circumference (MUAC)	Controls	30.66 ± 3.25	0.001
	IR	34.04 ± 4.87	
WC	Controls	89.17 ± 11.73	0.001
	IR	100.93 ± 14.55	
WHR	Controls	.829 ± 0.07	0.33
	IR	.840 ± 0.067	

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 2. Fasting blood sugar, insulin resistance and insulin in studied groups

Variables	Group	Mean ± SD	P value
FBG (mg/dL)	Controls	93.45 ± 33.61	0.49
	IR	97.84 ± 41.76	
Insulin (IU/ml)	Controls	10.3 ±4.9	0.05
	IR	16.7 ±5.1	
HOMA	Controls	3.3 ± 1.2	0.05
	IR	6.4 ± 2.5	

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

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-inflammatorystatus[13,14,15]. 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. [16]elucidatedthat fatty acid liberation from visceral fat into hepatocytes influencedas visceral fat mass augmented. This leads to elevated fatty acid in hepatocytes. Accordingly,stimulating synthesis and secretion of TGin the liver through its integration into TG-rich lipoproteins like very low-density lipoproteins (VLDLs)[17] circulating

Table 3. Liver functions and lipid profile in studied groups

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

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 (ìg/ml) in studied groups

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

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 augmenteddue to cumulatingvisceral 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 tables2 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 9fatty 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 ininitiation and activation of protein kinase C isoform (PKC-å) which leads to elevation of insulin receptor substrate-1(IRS-1) serine phosphorylation. Sequentially a reduction of IRS-1 tyrosine phosphorylation &IRS-1 related phosphatidyleinositol 3-kinase (PI3-K) activity causea reduction of insulin –stimulating glucose transport action[18] .

Contrarily, ALA improved insulin sensitivity viarising the responsibility of glucose transporter -4(GLUT-4),that leads to a development of glucose-6- phosphate[19]**.**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 radicals[22]**.**

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 dependenton the metabolism of FA and their endogenous synthesis. Also depends on intrauterine &prenatal programming and genetic variation [23].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 resistance[24].

Arachidonic acid (AA) acts as a powerful negative modulator of glucose uptake[25] and researches have elucidated elevated serum levels of arachidonic acid in diabetic subjects in comparison with normal controls[26].Thus, the data have been in agreement with teresearches that have shown a positive relationship between insulin resistanceandAA[20,21].

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

This work was corroborated by grant from National Research Centre, Egypt.

Conflict of interest

All authors declared that they have no conflict of interest.

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