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Effect of Oral Curcumin Administration on Insulin Resistance, Serum Resistin and Fetuin-A in Obese Children: Randomized Placebo-Controlled Study

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ABSTRACT

Obesity with insulin resistance in the pediatric population provides an increasing management challenge. This study investigated the effect of curcumin on serum resistin, fetuin –A and insulin resistance using the homeostasis model assessment of insulin resistance in obese children. A randomized, open-label and placebo-controlled study was carried out in the Pediatric Clinic of the National Research Centre. Thirty obese children divided randomly into two equal groups: a trial group and a placebo control group. Another group of 24 children with normal weight serving as controls for laboratory results. Each patient in the trial group received one capsule with the main meal containing 500 mg curcumin for 4 weeks. The control group received one capsule identical in color and size for the same duration. Obesity was shown to significantly elevate both resistin and fetuin-A serum levels and insulin resistance. A significant reduction in both resistin and fetuin-A was observed after curcumin supplementation (P<.010 & P<.011 respectively). They also exhibited significantly lower level of insulin resistance (P< .016). Short-term curcumin supplementation in obese children could decrease insulin resistance through reduction of high level of serum resistin and fetuin-A.

Key worlds: obesity, Visceral Fat, insulin resistance, resistin, fetuin-A, Curcumin

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INTRODUCTION

Obesity is a major obstacle in the maintenance of the human health system and causes various chronic diseases [1]. Recent evidence has shown that some dietary components such as spices may play a key role in the protection against and/or treatment of obesity and related metabolic disorders. Among these spices, turmeric has received considerable research interest because of its active ingredient Curcumin. Curcumin is the major yellow pigment extracted from turmeric, a commonly-used spice derived from the rhizome of the herb Curcuma longa [2]. It has been found to have antioxidant; antitumor, anti-inflammatory properties [3-5]. Curcumin can modulate various targets involved in obesity and metabolic diseases. Several reports suggest that curcumin has potential in prevention and treatment of obesity, diabetes, atherosclerosis, and metabolic syndrome [6].

An important link between obesity and diabetes is resistin. It is a protein hormone secreted by adipocytes, which contributes in insulin resistance (IR) in vivo and in vitro. It has been involved in the pathogenesis of obesity-mediated IR and type-2diabetes mellitus [7, 8]. Another important promoter of insulin resistance is Fetuin-A. It is a 64-kDa glycoprotein produced exclusively by the liver, not like other adipocytokines, which are derived from fat cells. Fetuin-A secreted into serum, it binds and inhibits the insulin receptor tyrosine kinase in skeletal muscle and hepatocytes. These cause insulin resistance in these target tissues. In humans, higher fetuin-A levels associate with obesity and insulin resistance. Also in patients with chronic kidney disease, end stage renal disease and associates with future risk of diabetes [9, 10]. Ismail et al., (2012) added that high serum fetuin-A level was found to be positively associated with metabolic syndrome [11].

Clinical trials of turmeric, curcumin or others of its bioactive components have been conducted in some studies. Sahebkar (2013) reported a systematic review and meta-analysis of randomized controlled trials investigating the effects of curcumin on blood lipid levels [12]. Mohammadi et al (2013) studied the effects of supplementation with curcuminoids on dyslipidemia in obese patients [13]. To the best of our knowledge, there is no study investigating the effects of curcumin on insulin resistance in obese children.

Our research mission is to facilitate progress towards breaking the link between exogenous obesity and its associated health conditions. The goal of this clinical research study was to learn if treatment of obese children with oral curcumin could decrease the insulin resistance by the down-regulation of Serum resistin and fetuin –A.

MATERIAL AND METHODS

Subjects:

The trial was approved by the local ethics committee of National Research Centre (NRC), Egypt. Written informed consent was obtained from parent of each child before enrolment. Thirty obese children, defined by BMI >95th percentile for age and sex met the following

eligibility criteria: age 10-18 years;, plus two risk factors: 1st or 2nd degree relative with diabetes, signs or conditions associated with IRS (Acanthosis nigricans; Hypertension; Dyslipidemia; Polycystic ovary syndrome) or metabolic syndrome(a cluster of increased waist circumference, dyslipidemia, impaired glucose metabolism, hypertension). They were enrolled between January and July 2013 at the National Research Centre, Pediatrics Clinic. Exclusion criteria in our study were medical conditions associated with obesity such as, hypothyroidism, Cushing syndrome or Turner syndrome, also obesity with mental retardation, such as Prader-Willi syndrome, Laurence-Moon- Biedl or subjects taking anti- inflammatory drugs . Another group of 24 children with normal weight serving as controls for laboratory results.

Study design:

A single center, randomized, open-label and placebo-controlled study was carried out in the Pediatric Clinic of the National Research Centre. Thirty obese children divided randomly into two equal groups: a trial group and a control group. Each patient in the trial group received one capsule with the main meal containing 500 mg curcumin for 4 weeks. The control group received one capsule identical in color and size for the same duration.

Study drug:

Curcumin was provided in a capsule form as a standardized powder extract obtained from Turmeric-Curcumin.com. It contains 500 milligrams per capsule of 95% standardized Curcumin extract .The extract is standardized to contain a minimum of 95% Curcuminoids: Curcumin, Demethoxycurcumin , Bisdemethoxycurcumin - the complete range in their natural composition ratio of 76:19:5. Thus, in our product the full spectrum of Curcumin antioxidant Curcuminoids are extracted from Turmeric (Curcuma longa root) and represented in their natural arrangement for maximum potency. This is the same material used in clinical trials and medical studies, free of added chemicals, 'enhancements', or treatments. Obese children ingested curcumin capsules (500 mg tab, once daily), for four weeks.

All patients included in this study were subjected to full history taking, thorough clinical examination, with emphasis on blood pressure measured according to American Heart Association guidelines, anthropometric indices: Body weight measured to the nearest 0.1 kg with a balance scale and height measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight divided by height squared (kg/m2). Waist circumference (WC) and hip circumference (HIP C) were measured.

Abdominal ultrasonography :

In addition to the routine abdominal ultrasound examination based on the clinical indication, ultrasonography (US) distinctively quantifies visceral fat and subcutaneous fat. We measured the maximum preperitoneal visceral fat thickness (VFT) and the minimum subcutaneous fat thickness (SFT) by US .The visceral fat thickness (VFT) was measured by 3.5-5 MHz convex –array probe .VFT is the distance between the internal surface of the abdominal

surface of abdominal muscle and the anterior wall of the aorta 1 cm above the umbilicus. The thickness of subcutaneous fat was measured by placement of a 7.5-MHz or 3.75-MHz probe perpendicular to the skin on the epigastrium. Longitudinal scans are obtained along the middle line (linea Alba). The thickness of the subcutaneous fat is defined as the distance between the anterior surface of linea Alba and the fat–skin barrier [14, 15] (Soo et al.,2004 & Wafaa et al.,2012).

The Laboratory measurements:

Fasting blood samples were collected on days 1 and 29. Blood samples were collected and centrifuged and separated serum was aliquotted into eppendorf, and stored at −80°C. FBS was assessed by an OLYMPUS AU 400 Chemistry Analyzer, Insulin level was estimated by Enzyme immunoassay (ELISA). Insulin resistance was calculated by the homeostasis model (HOMA-IR) using the following formula: HOMA-IR = fasting insulin (mU/l) × plasma glucose (mmol/l)/22.5. Resistin was measured using the BioVendor Human Resistin ELISA kit [15] (Sadashiv et al.,2012). Human fetuin-A was measured using ELISA technique with the kit provided from Adipo Bioscience, Inc. (2348 Walsh Ave, Suite C, Santa Clara, CA 95051, USA) [11] (Ismail et al., 2012).

Statistical analysis :

The standard computer program Statistical Package for the Social Sciences (SPSS) for Windows, release 12.0 (SPSS Inc., USA) was used for data entry and analysis. All numeric variables were expressed as mean ± standard deviation (SD). Comparison of different variables in various groups was done using Student t test for normal variable**.** To assess the effect of oral curcumin vs. placebo, the paired sample t test was used to compare means for normally distributed data. P values < 0.05 were considered as statistically significant.

RESULTS

Comparison between obese children and normal weight-matched controls:

Thirty-four obese children were referred to the study; four did not meet the inclusion criteria. A comparison between twenty four normal weight children and the obese group is shown in Table1. The mean age of the obese participants and normal weight controls was 11.28 ±3.71 and 11.45±3.44 years respectively with no statistical significant difference .The present work showed that HOMA-IR was significantly higher in obese children than in the normal weight controls (P = 0.001). Waist circumference, hip circumference, SCT, VFT and liver size were significantly higher in obese children than in the controls. Also, serum resistin and fetuin-A levels were significantly higher in obese children than in the controls shown in table 1.

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childrenTable1: Comparison between normal weight and obese.

	1=normal WT(n 24 children) 2= obese(n 30)	Mean	Std. Deviation	Sig. (2-tailed)
Age (years)	2	11.28	3.71	NS
	1	11.45	3.44	
SBP mmHg	2	112.65	14.37	NS
	1	107.00	11.54	
DBP mmHg	2	73.35	11.29	NS
	1	70.23	7.32	
WAIST C (cm)	2	92.99	14.69	.o4*
	1	90.52	16.30	
HIP C (cm)	2	104.66	19.04	,03*
	1	100.27	14.36	
SFT (cm)	2	1.8	0.4	0001.**
	1	1.2	0.2	
VFT(cm)	2	5.3	1.7	.0001**
	1	3.1	1.4	
Liversize (cm)	2	15.6	0.6	.01*
	1	13.6	0.4	
RESISTIN ng/ml	1	3.70	2.38	
	2	6.98	4.24	.004**
FETUIN-A ug/ml	1	204.72	93.55	
	2	295.35	91.54	.024
HOMA-IR	1	2.97	1.42	.001**
	2	3.75	4.65	

. * P value is significant. ** P value is highly significant
(SBP) Systolic blood pressure, (DBP) diastolic blood pressure
SFT) Subcutaneous fat thickness, (VFT) visceral fat thickness)
HOMA-IR=the homeostasis model assessment

Results of the trial:

Thirty obese children divided randomly into two equal groups: a trial group and a control group. Each patient in the trial group received one capsule with the main meal containing 500 mg curcumin for 4 weeks. The control group received one capsule identical in color and size for the same duration. Outcome measures were the homeostasis model assessment insulin resistance (HOMA-IR), serum resistin and fetuin –A. Out of fifteen obese children randomized to take curcumin, only fourteen had completed the study; (One was lost to follow up). On other hand, only 11 Participants taking placebo had completed the study; {Lost to follow up (n=2), School commitments (n=2)}. Curcumin capsules were well tolerated. Adherence to therapy based on pill counts for the fourteen participants was 100 %.

Table 2 shows comparison between basal data of curcumjn and placebo groups. No statistically significant differences were detected regarding anthropometric and biochemical results.

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Table 2: Comparison between basal data of curcumjn and placebo groups.

	ID	N	Mean	Std. Deviation	Sig. (2-tailed)
AGE(years)	2	14	15.5714	5.79740	NS
	3	11	16.5357	8.69674	
HIPC cm	2	14	113.1071	11.70171	NS
	3	11	108.7143	12.71842	
WC cm	2	14	98.2857	12.63803	NS
	3	11	95.6429	11.33990	
BMI	2	14	35.3714	6.32597	NS
	3	11	32.6014	6.29557	
WHTR	2	14	.6393	.07416	NS
	3	11	.6171	.06696	
WHR	2	14	.8700	.09430	NS
	3	11	.8821	.05466	
HOMAIR-IR	2	14	3.58	1.34	NS
	3	11	3.00	.98	
Resistin ng/ml	2	14	6.63	3.72	NS
	3	11	7.17	5.13764	
Fetuin-A ug/ml	2	14	298.93	71.08	NS
	3	11	282.50	104.65	

(SBP) Systolic blood pressure, (DBP) diastolic blood pressure
(SFT) Subcutaneous fat thickness, (VFT) visceral fat thickness
HOMA-IR=the homeostasis model assessment

Oral curcumin effect on anthropometry and body composition:

Comparison of samples taken immediately before dosing and at 29 day was performed by paired t test for individual values are shown in table 3. Whereas Oral curcumin therapy resulted in a tendency to reduction in body weight this was not statistically significant (P= 0 .064). A beneficial treatment effect of Oral curcumin over placebo was found for visceral fat (P=.04).

Oral curcumin treatment effect on parameters of Insulin resistance:

Oral curcumin had a beneficial treatment effect over placebo for serum resistin and fetuin-A. Our results revealed that there were statistically significantly decrease between samples taken immediately before dosing and at 29 day as regards serum resistin and fetuin – A(P= .010 and.011respectivly) as shown in Table 4 and fig1,2. There was significant statistical beneficial treatment effect of oral curcumin (P= .016) over placebo for Insulin resistance estimated by the Homeostasis Model Assessment of IR (HOMA-IR).

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Table 3: Paired Samples Test for anthropometry and ultrasonography of curcumjn and placebo groups

Oral Curcumin		NO	Mean	Std. Deviation	Paired Differences Mean	SD	Sig. (2-tailed)
Pair 1	WT	14	78.28	21.54	2.6462	4.68359	NS
	WT2	14	75.64	19.72			
Pair 2	WC	14	93.65	13.80	.8846	12.21705	NS
	WC2	14	92.77	8.85			
Pair 3	HIPC	14	108.31	14.07	1.15	7.15	NS
	HIPC 2	14	107.15	12.95			
Pair 4	VFT	14	5.04	1.24	1.0575	.0332	.045
	VFT2	14	3.99	0.81			
Pair 5	SCF	14	2.42	0.58	.2063	.616	NS
	SCF2	14	2.22	0.62			
Pair 6	LIVER SIZE	14	14.20	1.23	.3975	.63107	NS
	LIVER SIZE 2	14	13.81	0.97			
Placebo		NO	Mean	S D	Paired Differences Mean	SD	Sig. (2-tailed)
Pair 1	WT	11	87.7273	11.05523	.4000	1.28452	NS
	WT2	11	87.3273	11.28575			
Pair 2	WC	11	99.6364	6.51188	1.63207	.49209	NS
	WC2	11	97.9545	6.30260			
Pair 3	HIPC	11	112.0000	8.61394	1.74773	.52696	NS
	HIPC 2	11	111.6364	8.76667			
Pair 4	VFT	11	4.01	1.52	- .0182	.0766	NS
	VFT2	11	4.10	1,23			
Pair 5	SCF	11	2.31	.44	.0164	.07619	NS
	SCF2	11	2.30	.44			
Pair 6	LIVER SIZE	11	14.88	1.47109	0	0	NS
	LIVER SIZE 2	11	14.86	1.47109			

Table 4: Paired Samples Test for biochemistryof curcumjn and placebo groups

Oral curcumin		NO	Mean	SD	Paired Differences Mean	SD	Sig. (2-tailed)
Pair 1	RESISTIN	14	6.63	3.72	2.77	3.60	.010
	RESISTIN 2	14	3.85	1.60			
Pair 2	FETUIN-A	14	298.93	71.08	62.26	82.46	.011
	FETUIN -A2	14	236.67	66.85			
Pair 3	HOMA-IR	14	3.58	1.34	4.09	3.46	.016
	HOMA-IR 2	14	2.36	1.49			
Placebo		NO	Mean	SD	Paired Differences Mean	SD	Sig. (2-tailed)
Pair 1	RESISTIN	11	7.17	5.13	-.2545	.40	NS
	RESISTIN 2	11	7.42	5.38			
Pair 2	FETUIN-A	10	282.50	104.65	.9000	9.57	NS
	FETUIN -A2	10	281.60	102.50			
Pair 3	HOMA-IR	11	3.00	.98	-.0545	.40	NS
	HOMA-IR 2	11	3.05	1.01			

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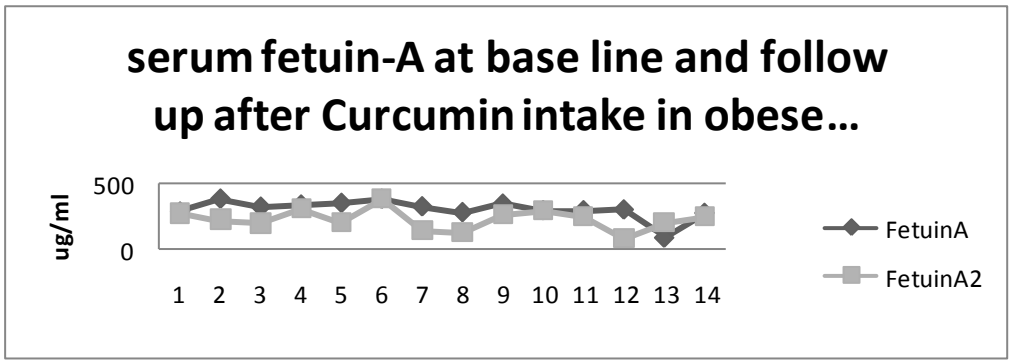

Figure 1: serum fetuin-A at base line and follow up after Curcumin intake in obese children.

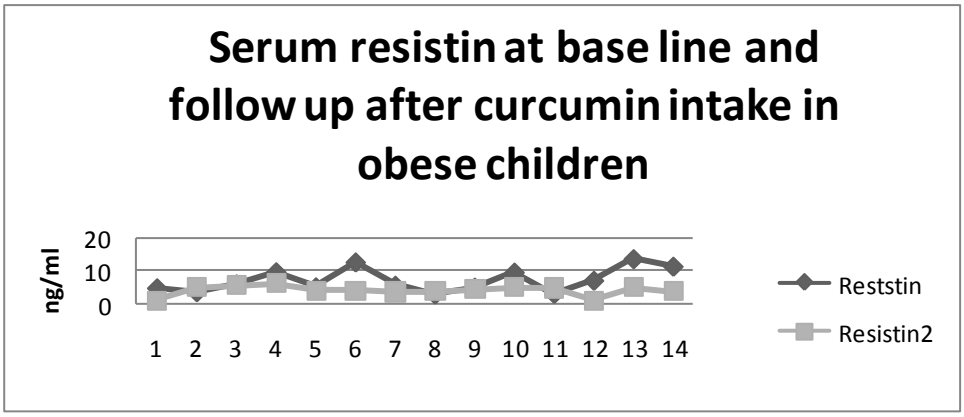

Figure 2: Serum resistin at base line and follow up after curcumin intake in obese children.

DISCUSSION

In obesity, there are higher circulating concentrations of inflammatory cytokines than in lean beings, and it is believed that they play a role in causing insulin resistance. The main source of pro-inflammatory cytokines in obesity is the adipose tissue; they are mainly produced by infiltrating macrophages, although adipocytes play a role. In this way, blood concentrations of these cytokines are lowered following weight loss [16- 18]. Obesity is closely linked to insulin resistance. Energy metabolism is primarily controlled by insulin, a hormone that promotes the synthesis and storage of proteins, carbohydrates, and lipids. Thus, insulin resistance is commonly associated with obesity.

Our findings showed a significant increase of serum resistin in our obese children. Initial studies showed that resistin was up-regulated in rodent model of obesity and IR [19-20] . Some investigations of human resistin in relation to obesity have shown higher serum resistin levels in obese subjects compared with lean subjects [21].Recently, Habib. 2012 found that diabetic patients in Saudi Arabia have significantly higher resistin levels that were positively correlated

with body fat mass. He cited that this was supporting the evidence that resistin plays an important role in the pathogenesis of obesity and insulin resistance [22].

Visceral abdominal fat is a risk factor for obesity-related complications, and there is increasing evidence that abdominal adiposity may be a contributing factor to complications [23 ,24] . Our study showed a significant increase of visceral fat in obese children. Studies in human subjects have highlighted increased resistin expression in adipose tissue particularly abdominal depots [25-26].

As regards serum fetuin–A level a significant increase was detected in our obese children. Our result is in accordance with the results of other studies showing elevated serum fetuin-A levels in obese patients such as Ismail et al (2012) who reported that fetuin-A levels were higher in adults and children with obesity and related to insulin resistance [11].

Turmeric has received considerable research interest because of its active ingredient, curcumin (CCM). CCM interacts with specific proteins in adipocytes, pancreatic cells, hepatic stellate cells (HSC), macrophages, and muscle cells, where it suppresses several cellular proteins such as transcription factor nuclear factor-kappaB (NF-κB), signal transducer and activator of transcription (STAT)-3, and Wnt/β-catenin and activates peroxisome proliferator-activated receptor (PPAR)-γ and nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) cell signaling pathway. In addition, curcumin downregulates the inflammatory cytokines, resistin and fetuin-A [27].

This study demonstrates that oral curcumin for obese children is safe and well tolerated as no nausea or gastrointestinal upset is recorded. It has a beneficial effect on visceral abdominal fat as it causes a significant decrease of VFT. Visceral abdominal fat is implicated in the development of insulin resistance in adolescents [28, 29] , and loss of visceral, rather than subcutaneous fat in obese children has greater metabolic benefits. We found that 4 weeks of oral curcumin resulted in significant improvement in insulin resistance and significant down-regulation of the serum levels of resistin and fetuin-A.

This study had some limitations. First, we did not follow-up our cases to assess the persistence of the drug effect after its cessation. Second, this study did not analyze the role of puberty although it may affect IR.

In conclusion, curcumin could decrease insulin resistance in obese children through down- regulation of serum resistin and fetuin-A and reducing visceral fat, suggesting that curcumin has potential in prevention of obesity associated metabolic diseases.

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