World Journal of Medical Sciences 7 (2): 100-104, 2012
ISSN 1817-3055

DOI: 10.5829/idosi.wjms.2012.7.2.1104

Potent Role of Lipocalin in Childhood Obesity

[1]Hanaa H. Ahmed, [2]Enas R. Abdel Hameed, [1]Aziza B. Shalby and [2]Hala G. El-Nady

[1]Department of Hormones, National Research Centre, Dokki, Giza, Egypt
[2]Department of Child Health, National Research Centre, Dokki, Giza, Egypt

Abstract: The current study is undertaken to investigate the potential role of lipocalin in the low grade inflammation associated with obesity in children. This study was conducted on 100 children (age range from 8-12 years) divided into two groups, the first group included 50 obese children and the second group included 50 lean controls. Anthropometric measurements including weight, height and body mass index were recorded. Plasma levels of insulin, cholesterol, triglycerides, high sensitivity C-reactive protein, interleukin-6 and lipocalin-2 (LCN-2) were determined. The present results reveal that plasma lipocalin concentration was significantly increased in obese children when compared with the lean controls. Moreover, circulating lipocalin-2 concentration showed significant positive correlation (r=0.850) with adiposity (BMI), hypertriglyceridemia (r=0.607) and hypercholesterolemia (r=0.544). In addition, there was a strong positive association between LCN-2 concentration and high sensitivity C-reactive protein and interleukin -6 in obese children. This association suggested that LCN-2 has a positive involvement in the low-grade chronic inflammation accompanying obesity. The present study reveal that LCN-2 is an inflammatory marker closely related to obesity in children .Measurement of plasma LCN-2 might be useful as perspective indicator for evaluating the outcomes of various clinical interventions for obesity -related metabolic diseases.

Key words: Lipocalin · Childhood obesity · Inflammation · Lipid profile

INTRODUCTION

Obesity is the most common risk factor for insulin resistance, type 2 diabetes mellitus, (T2DM) [1]. Although the detailed molecular events that link obesity with it's associated pathologies are not well understood, a growing body of evidence suggests that systematic inflammation might be an important mediator [2]. Studies have demonstrated close associations between obesity and a state of low-grade chronic inflammation characterized by macrophage infiltration in adipose tissue and increased circulating concentrations of proinflammatory molecules, including acute phase proteins, cytokines and chemokines [3,4]. Lipocalin-2 is a secretory glycoprotein member of the highly heterogenous family of lipocalins. It is a component of the innate immune system with a key role in the acute -phase response to infection [5]. LCN-2 also known as neutrophil gelatinase-associated lipocalin (NGAL) or siderocalin was oringinally identified as a component of neutrophil granules. LCN-2 expression has been observed in most tissues and its synthesis is induced in epithelial cells during inflammation [6]. Recent reports have described this protein as an adipokine closely related to insulin resistance [7]. Furthermore, the high circulating levels of LCN-2 have been described in adult obese being associated with anthropometric variables and measures of insulin resistance [8].

Lipopolysaccharide strongly stimulates LCN-2 expression in adipose tissue and liver, strengthening its role as an acute-phase reactant [9].Furthermore, the expression of LCN-2 in adipocyte is strongly induced by the pro-inflammatory cytokine. Since obesity is considered a state of low- grade chronic inflammation [10], the implication of LCN-2 in the inflammatory signaling pathways activated in obesity seems plausible. In this study, we analyzed the influence of obesity on the circulating level of LCN-2 in children. Present study also, is extended to evaluate the hypothesis that LCN-2 operates as a modulator of inflammation in childhood

Corresponding Author: Hanaa H. Ahmed, Department of Hormones, National Research Centre, Dokki, Giza, Egypt

obesity as we studied it's relation to circulating proteins involved in inflammation in a well-characterized group of obese children comparing with the lean controls.

SUBJECTS AND METHODS

A total number of 100 children (age range 8-12 years) were enrolled in the current study. The subjects of this study were selected on the basis of their body mass index (BMI) and included: group (I) 50 obese (29 males and 21 females cases) (BMI ≥ 30kg/m^2) and group (II) 50 lean control (BMI < 25kg/m^2) (matched for age and sex). BMI was calculated as weight in kilograms divided by the square of height in meters. The protocol was approved by the Ethical Committee for Medical Research -Dokki, Cairo Egypt. Five milliliter blood samples from both groups (cases and controls) were collected by venipuncture after an overnight fast in EDTA containing tubes. Blood samples were centrifuged in a cooling centrifuge at 4°C for 10 min. to obtain plasma. Plasma insulin was analyzed by mean of an enzyme-amplified chemilumi-nescence assay (IMMULITE, Diagnostic products Corp. Los Angeles, CA, USA) according to the method described by Eastham [11]. Total cholesterol and triglycerides levels were determined calorimetrically using a Stanbio Laboratory Kit (USA), according to the method of Richmond [12] and Drayer [13], respectively. High sensitivity C-reactive protein (CRP) concentration was determined by using a microplate immune enzymetric assay provided from Monobind INC. USA [14], while Interleukine-6 concentration was quantified using a commercially available ELISA kit (Ray Biotech, Inc. USA) according to the method of Bauer and Hermann[15]. Plasma lipocalin LCN-2 level was estimated using commercially available ELISA kits (R&D systems Europe Ltd, Abingdon, UK) according to Kjeldsen *et al.* [16] procedure.

Statistical Analysis: Data in the present work are presented as means ± standard deviation (Means ± SD). Differences between the groups were assessed by two-tailed unpaired student's t test. The descriptive frequencies of the variables were adequate for the use of chi-square, cross-tabulation test. Pearson's correlation coefficient (r) was used to analyze the association between variables. The calculations were performed using the (SPSS, Chicago, II, USA) and $P < 0.05$ was considered statistically significant.

RESULTS

The results in Table 1 show the clinical characteristics of the study participants. The statistical analysis of the data in this table show significant increased in obese children as compared to the lean controls ($P < 0.05$). Data in Table 2 revealed that plasma total cholesterol, triglycerides and insulin are significantly higher in obese children as compared to the lean controls ($P < 0.05$). The circulating levels of the inflammatory marker CRP and interleukin-6 show significant increase in group I obese children as compared to group II lean controls ($P < 0.05$). Significant elevation in plasma level of lipocalin 2 (LCN2) is detected in obese children when compared to the lean control one ($P < 0.05$). Lipocalin-2 level shows significant positive correlation with insulin ($r = 0.912$, $P < 0.01$), the inflammatory marker CRP ($r = 0.960$, $P < 0.01$) and IL-6 ($r = 0.976$, $P < 0.01$). Also, it declares demonstrates significant positive correlation with both cholesterol ($r = 0.544$, $P < 0.01$) and triglycerides ($r = 0.607$, $P < 0.01$) as shown in Table 3. Moreover, LCN-2 shows significa nt positive correlation with BMI ($r = 0.850$, $P < 0.01$). Also, CRP and interleukin-6, reveal significant positive correlation with BMI ($r = 0.879$ and 0.882 respectively) at $P < 0.01$ (Table 3). The relation between LCN-2 and BMI by cross tabulation is shown in Table 4. It reveals a sensitivity amounting to 87.5% and specificity reached to 82.5%. The statistical significant difference between LCN-2 and BMI is detected by Fisher's Exact test ($P < 0.05$).

Table 1: Clinical characteristics of the study subjects

Parameters	Group I (n =50) Mean ± SD	Group II (n =50) Mean ± SD	Sig. (2-Tailed)
Age (years)	10.91± 2.2	10.43 ± 2.5	0.32
Sex	1.42 ± 0.49	1.55 ± 0.5	0.19
Weight (Kg)	60.41 ± 14.46	33.57 ± 11.94	0.00
Height (m)	141.47 ± 13.05	135.13 ± 13.73	0.02
BMI (Kg/m^2)	29.75 ± 3.31	17.77 ± 2.75	0.00

Table 2: Demographic data of the studied groups.

Parameters	Group I (n =50) Mean ± SD	Group II (n =50) Mean ± SD	Sig. (2-Tailed)
Chol. (mg/dl)	174.71 ± 9.8	163.69 ± 9.3	0.00
TG. (mg/dl)	98.81 ± 12.2	84.43 ± 7.5	0.00
Insulin (μIU/ml)	26.32 ± 6.0	9.98 ± 3.07	0.00
CRP (μg/ml)	2.63± 0.18	0.74 ± 0.11	0.00
IL-6 (pg/ml)	4.11± 0.18	1.12± 0.11	0.00
LCN2 (ng/ml)	47.27 ± 5.20	17.75 ± 3.19	0.00

Table 3: Correlation between the different studied parameters in obese children.

Parameters	Correlations	Insulin	CRP	Chol	TG	IL-6	LCN2	BMI
Insulin	Pearson correlation	1	0.900**	0.674**	0.669**	0.905**	0.912**	0.746**
	Sig. (2-tailed)	-	0.000	0.000	0.000	0.000	0.000	0.000
CRP	Pearson Correlation	0.900**	1	0.570**	0.633**	0.990**	0.960**	0.879**
	Sig. (2-tailed)	0.000	-	0.000	0.000	0.000	0.000	0.000
Chol	Pearson Correlation	0.674**	0.570**	1	0.743**	0.534**	0.544**	0.437**
	Sig. (2-tailed)	0.000	0.000	-	0.000	0.000	0.000	0.000
TG	Pearson Correlation	0.669**	0.633**	0.743**	1	0.606**	0.607**	0.503**
	Sig. (2-tailed)	0.000	0.000	0.000	-	0.000	0.000	0.000
IL-6	Pearson Correlation	0.905**	0.990**	0.534**	0.606**	1	0.976**	0.882**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	-	0.000	0.000
LCN-2	Pearson Correlation	0.912**	0.960**	0.544**	0.607**	0.976**	1	0.850**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	-	0.000
BMI	Pearson Correlation	0.746**	0.879**	0.437**	0.503**	0.882**	0.850**	1
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000	-

Table 4: Cross tabulation between lipocalin-2 and BMI in obese children.

			BMI		
Parameter	Cut-off	Count %	More than or equal to cut-off	Less than cut-off	Total
LCN2	More than or equal to cut-off	Count % within LCN2	49	7	56
			87.5%	12.5%	100.0%
	Less than cut-off	Count % within LCN2	7	33	40
			17.5%	82.5%	100.0%
Total	Count % within LCN2		56	40	96
			58.3%	41.7%	100.0%

DISCUSSION

Obesity related dyslipidemia, chronic inflammation and oxidative stress were associated with atherosclerotic sequels [17]. Adipose tissue acts as an active autocrine, paracrine and endocrine organ, secreting an increasing number of adipokines that participate in diverse metabolic processes [18]. In this sense, LCN-2 has been recently described as an adipokine involved in inflammation and insulin resistance [6]. The main finding of this study was that the circulating LCN-2 level is upregulated in obese children. Also, plasma LCN-2 showed a strong correlation with the well established pro-inflammatory markers (CRP and IL-6). It has been found that obesity is strongly associated with high sensitivity C-reactive protein (HsCRP) concentration and the inflammatory marker TNF-α does not directly induce HsCRP, but can potentiate the induction of HsCRP by stimulating the synthesis of IL-6 [19]. The increased serum level of LCN-2 in adult obesity has been previously reported, where upregulated expression levels have been detected in adipose tissue and serum [3]. The present study provides evidence that obese children exhibited high circulating level of LCN-2, thus, contrasting to the previous published data in human models of obesity [20] and in contrary to other studies with no detected increase in LCN-2 level in human obesity [21].

Development of obesity is associated with modification in adipose tissue involving adipogenesis, angiogenesis and proteolysis of the extracellular matrix. Moreover, the basement membrane of adipocytes has to be degraded in order to allow the hypertropic development observed in obesity [22]. The present findings of enhanced level of plasma LCN-2 in obese

group as well as the significant positive correlation with BMI indicate the indirect active role of LCN-2 in obesity progression. Chronic low-grade inflammation is known to be a mediator in the development of obesity-related disease. In agreement with previous results, the present data showed an increase in the plasma levels of some key of pro-inflammatory agents such as, CRP and interleukin-6 in obese children [23]. Also, in this study we established a significant association of these markers with increased level of LCN-2 in a positive significant manner. This fits with the same recorded by Wang *et al.* [3] as they observed an association between serum LCN-2 and CRP (both increased in obese adults). Thus, the current data suggested the implication of LCN-2 in low-grade inflammation associated with obesity in children. These matched results raise the question whether LCN-2 has causal effects on insulin resistance, hyperglycemia or inflammation associated with obesity and if it could, thus it might be a treatment target. Hence, it could be concluded that, plasma LCN-2 level is increased in obese children and it showed a positive correlation with the different pro-inflammatory markers. This highlights its involvement in the low-grade chronic inflammation accompanying obesity.

REFERENCES

1. Janke, J., S. Engeli, M. Boschmann, F. Adams, J. Böhnke and F.C. Luft, A.M. Sharma and J. Jordan, 2006. Retinal Binding Protein 4 in human obesity. Diabetes, 55: 2805-2810.
2. Van, L.F., I.L. Mertens and C.E. DeBlock, 2006. Mechanisms linking obesity with cardiovascvular disease. Nature, 444: 875-880.
3. Wang, Y., K.S. Lam, E.W. Kraegen, G. Sweeney, J. Zhang, A.W. Tso, W.S. Chow, N.M. Wat, J.Y. Xu, R.L. Hoo and A. Xu, 2007. Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance and hyperglycemia in humans. Clin. Chem., 53: 34-41.
4. Fruhback, G., 2008. Overview of adipose tissue and its role in obesity and metabolic disorders. Methods Mol. Biol., 456: 1-22.
5. VanDam, R.M. and F.B. Hu, 2007. Lipocalin and insulin resistance: etiological role of retinol-binding protein 4 and lipocalin -2. Clin. Chem., 53: 5-7.
6. Kjeldsen, L., 2000. Lipocalins. Biochem. Biophys. Acta, 1482: 272.
7. Tan, K., R. Adya, X. Shan, F. Syed and H. Randeva, 2009. *Ex vivo* and *in vivo* regulation of lipocalin-2, a novel adpokine, by insulin. Diabetes Care, 32: 129-131.
8. Greenberg, A.S. and M.S. Obin, 2006. Obesity and the role of adipose tissue in inflammation and metabolism. Am. J. Clin. Nutr., 83(2): 461S-465S.
9. Aigner, F., H. Maier, H. Schwelberger, E.A. Wallnöfer, A. Amberger, P. Obrist, T. Berger, T.W. Mak, M. Maglione, R. Margreiter, S. Schneeberger and J. Troppmair, 2007. Lipocalin-2 regulates the inflammatory response during ischemia. Am. J. Transplant, 7: 779-788.
10. Badman, M.K. and J.S. Flier, 2007. The adipocyte as an active participant in energy balance and metabolism. Gastroenterology, 132: 2103-2115.
11. Eastham, R.D., 1985. Biochemical Values in Clinical Medicine. 7th ED. Bristol. England. John Wrights & Sons, ITD.
12. Richmond, W., 1973. Preparation and properties of a cholesterol oxidase from *Nocrdia* sp and its application to the enzymatic assay of cholesterol in serum. Clin. Chem., 19: 1350-1356.
13. Drayer, R.L., 1970. Fundamental of Clinical Chemistry. NW Teitz, Ed. WB Saunders, Philadelphia, pp: 329.
14. Kimberly, M.M., H.W. Vesper, S.P. Caudill, G.R. Cooper, N. Rifai, F. Dati and G.L. Myers, 2003. Standardization of immunoassays for measurement of high-sensitivity C-reactive protein phase I. Clin Chem., 49: 611-616.
15. Bauer, J. and F. Hermann, 1991. Interleukin -6 in clinical Medicine. Hematology, 62: 203-210.
16. Kjeldsen, L., A.H. Johnson and H. Sengelov, 1993. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J. Biol. Chem., 268: 10425-10432.
17. Zelzer, S., N. Fuchs, G. Almer, R.B. Raggam, F. Prüller, M. Truschnig-Wilders, W. Schnedl, R. Horejsi, R. Möller, D. Weghuber, R. Ille and H. Mangge, 2011. High density lipoprotein cholesterol level is a robust predictor of lipid peroxidation irrespective of gender, age, obesity and inflammatory or metabolic biomarkers. Clin. Chim. Acta. 412: 1345-1349.
18. Trayhurn, P., 2007. Adipocyte biology. Obes. Rev., 8(Suppl 1): 41-44.

19. Lao, X.Q., G.N. Thomas, C.Q. Jiang, W.S. Zhang, P. Adab, T.H. Lam and K.K. Cheng, 2010. Obesity, high-sensitive C-reactive protein and snoring in older Chinese: The Guangzhou Biobank Cohort Study. Respir. Med., 104: 1750-1756.
20. Lin, Y., M.W. Rajala, J.P. Berger, D.E. Moller, N. Barzilai and P.E. Scherer, 2001. Hyperglycemia-induced production of acute phase reactants in adipose tissue. J. Biol. Chem., 276: 42077-42083.
21. Yan, W., Q. Yang, N. Mody and C.H. Hsu, 2007. The adipokine lipocalin 2 is regulated by obesity and promote insulin resistance. Diabetes, 56: 2533-2540.
22. Catalán, V., J. Gómez-Ambrosi, A. Rodríguez, B. Ramírez, C. Silva, F. Rotellar, M.J. Gil, J.A. Cienfuegos, J. Salvador and G. Frühbeck, 2009. Increased adipose tissue expression of lipocalin 2 in obesity is related to inflammation and matrix metalloproteinase-2 and metalloproteinase 9 in humans. J. Mol. Med. (Berl), 87: 803-813.
23. Tauman, R., L.M. O'Brien and D. Gozal, 2007. Hypoxemia and obesity modulate plasma C-reactive protein and interleukin-6 levels in sleep-disordered breathing. Sleep Breath. 11: 77-84.