ORIGINAL ARTICLE

Pro-Inflammatory Profile of Extremely Obese Children in a Local Population of Central Punjab, Pakistan

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ABSTRACT

Objective: To evaluate the status of pro inflammatory markers in children with extreme obesity.
Subjects and methods: 37 children were recruited for the study (20 non obese and 17 obese). Serum concentration of C reactive protein (CRP), α-2 macroglobulin (A2M) and hepatoglobin were measured. Anthropometric findings were correlated with biochemical parameters.
Results: Mean serum CRP, A2M and hepatoglobin were several fold higher in obese group as compared to the controls (p value < 0.05). CRP was significantly correlated with body weight (BW), body mass index (BMI) and A2M (p value <0.05).
Conclusion: Childhood obesity is associated with a pro-inflammatory state before the appearance of co-morbidities of metabolic syndrome, where as CRP, A2M and haptoglobin are the most likely indicator of development of inflammation.
Keywords: CRP, obese children, inflammatory profile

INTRODUCTION

The prevalence of obesity the global epidemic has reached alarming levels, with more than 1 billion overweight adults of which 300 million are considered as clinically obese. Worldwide, over 22 million children under the age of 5 are severely overweight, as are 155 million children of school age. This implies that one in 10 children worldwide is overweight. It was estimated in 2000 that more than half of US adults were overweight reflecting an increase of 61% within 10 years[1].

Pakistan is a developing country with the prevalence of obesity comparable to some of the developed countries. Various indigenous studies have documented the status of overweight and obesity with associated complications, in Pakistan[2]. Despite this fact, these investigations have not targeted the real picture, especially in children.

Recent studies carried out in during the last two decades demonstrate that obesity is a metabolic disorder, which might be due to dysfunction of single gene (monogenic obesity) or numerous genes, each of these making up a minor contribution in determining the phenotype of obesity (polygenic obesity)[3]. Parental obesity along with early menarche of mother, contributes to childhood obesity[4]. Obesity due to fat deposition in visceral region of body i.e. central or abdominal obesity is more prone to health issues and has greater risk of metabolic syndrome than peripheral or subcutaneous obesity[5].

Obesity is the direct result of an imbalance between energy intake and energy expenditure or an abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired. This storage is controlled by different complex mechanisms which include the interplay of environment with genetic, neuronal and biochemical interactions[6].

The excess energy is primarily stored in adipose tissue in the form of triglycerides. Although adipocytes are specifically designed to store energy and easily fill up with fat but morphological changes are associated with adipose tissue growth[7]. In response to adipocyte hypertrophy during development of obesity, the function of adipose tissue is compromised.

In recent years, it has become clear that obesity also gives rise to a heightened state of inflammation. The link between obesity and inflammation was first established when a positive correlation between adipose mass and expression of the pro inflammatory gene tumor necrosis factor-α (TNFα) was reported[8]. The link between obesity and inflammation has been further illustrated by the increased plasma levels of several pro-inflammatory markers including cytokines and acute phase proteins like C-reactive protein (CRP) in obese individuals[9,10].

Although increased visceral fat depots[11] and adipocyte hypertrophy[7] had been linked to a higher degree of adipose inflammation, until recently, the exact pathways leading to a pro-inflammatory state of adipose tissue in obese individuals remained

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unidentified. However, recently, much attention has been diverted to the role of macrophages. In 2003, two studies indicate that diet-induced obesity is associated with infiltration of macrophages into white adipose tissue[12]. Infiltrated macrophages, which are part of the stromal vascular fraction of adipose tissue, are subsequently responsible for the production of a wide variety of pro-inflammatory proteins including, CRP, serum amyloid A, TNFα, and interleukin-6 (IL-6) The release of first line of pro-inflammatory proteins (IL-6, TNF α etc.), trigger the secretion of hepatocytic acute phase proteins like CRP, haptoglobulin and α-2 macroglobulin.

The proposed study envisages targeting a simultaneous measurement of levels of pro-inflammatory markers (CRP, alpha-macroglobulin and haptoglobin) in children (lean and obese), using multiplex system. Multiplexed protein analysis may serve as tool for advance diagnosis of metabolic diseases with complicated clinical presentations. The technique is based on primary antibodies as mobilized probes on solid surfaces, whereas protein antigens are labeled by various flourophores along with secondary antibodies, for precise detection. By recognizing biochemical variations that predispose to obesity through different mechanisms, one may be able to classify obese subjects into subgroups that might have particular beneficial responses to specific diets and/or exercise regimes, drugs or surgery.

In summary, obesity represents a major health threat, and evidence based effective therapies to minimize obesity-related co-morbidities are needed.

MATERIALS AND METHODS

The present study based on a total of 37 children, 0.5-10 years of age, was approved by the Ethical Committee of the Centre for Research for Molecular Medicine (CRiMM), The University of Lahore (UOL). Participation of the subjects in this study was voluntary and written informed consent to participate in the study was obtained in each case. The subject and/or his/her parents were informed about the potential benefits and risks of this study. The subjects underwent a detailed medical examination by a competent physician and a questionnaire including information about family origin, family history of diabetes and obesity, acanthosis nigricans, lifestyle and eating habits, was completed. All the subjects were physically examined by the investigator for any growth and developmental anomalies and for recording anthropomorphic data. Patients with syndromic obesity (e.g. Cushing's syndrome, Down syndrome, autism amongst others) were excluded from the study. History of diabetic condition in grandparents or other second degree relatives of subjects, if available, was also recorded. This is a cross-sectional prospective study. On the basis of BMI, the Subjects were divided into the following 2 age-matched groups:

Group I: Control: children with a BMI <80th percentile (n=20, mean age: 4.64 years)

Group II: Obese: children with a BMI >95th percentile (n= 17; mean age: 7.46 years)

Body weight (BW) and height were recorded for all patients. Body mass index (BMI) was calculated according to the equation: BMI = BW (kg)/height (m)^2

BMI percentile was determined was based on WHO growth charts. Three to four ml of venous blood was drawn from the cubital vein between 11:00-13:00 hours after overnight fasting of 12 hours. The blood samples were centrifuged at 5,000 rpm and the serum sample was aliquot and stored at -20^o C until used. All biochemical parameters were determined in duplicate using standard procedures. The serum levels of C reactive proteins (CRP), haptoglobin and alpha 2 macroglobin were estimated by Bioplex analyzer System using acute phase Inflammatory marker panel (Bio-Rad Laboratories, Hercules, CA, USA). The data collected was analyzed using SPSS version 17 (SPSS, Inc, Chicago, IL, USA). The significance of difference between control and obese subjects was analyzed by 2-tailed Student's t test. Pearson test was used to calculate correlation between variables of interest. P value < 0.05 was considered statistically significant.

RESULTS

The physical characteristics of subjects are summarized in Table 1 and Fig. 1. The non-obese children (n=20, mean age: 4.64 years) had a mean BMI of 18.18 compared to 28.71 kg/m^2 in obese subjects (n=17, mean age: 7.46 years). The mean weight and height of the non-obese subjects were 17.58 kg and 0.97 m compared to 46.31kg and 1.23 m in the obese group.

Table 1: Physical characteristics of children (0.5-10 year old). Data are expressed as mean±SEM (median).

Group	Non-obese	Obese	P
N	20	17	
Weight (kg)	17.58±1.79 (15.75)	46.31±6.13 (45)	0.000[a]
Height (m)	0.97±0.04 (0.98)	1.23±0.06 (1.30)	0.001[a]
BMI (kg/m^2)	18.18±1.33 (16.97)	28.71±1.80 (26.63)	0.000[a]

[a]Significantly different from non-obese group (Student's t-test);

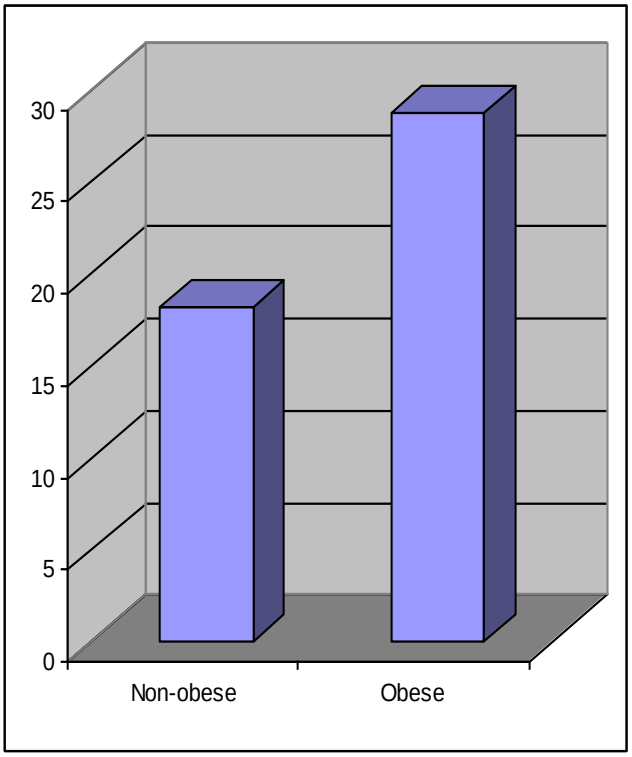

Fig 1: BMI values in non-obese and obese children (0.5-10 year old).

In both groups age, BW and Height were highly correlated with each other ($p<0.01$).

Biochemical Profile: The serum levels of CRP, α-2macroglobulin and haptoglobin are shown in Table 2

Table 2: Serum α-2macroglobulin and CRP concentration in non-obese and obese subjects (0.5-10 year old). Data are expressed as mean ± SEM (median).

Group	Non-obese (20)	Obese (17)	P
Serum concentration α-2macroglobulin (g/l)	1.15±0.21	2.88±0.72	<0.05[a]
Serum CRP concentration (µg/ml)	0.53±0.16	4.07±0.98	<0.05[a]

[a]Significantly different from non-obese group (Student's t-test).

CRP: The mean serum CRP concentrations in the obese group were several fold higher compared to the controls (0.53 vs. 4.07; $p<0.05$). CRP levels ranged from 0-2.75 (0.36) and 0-12(1.88) in non obese and obese children respectively. CRP was found highly correlated with BW and BMI in obese ($r=0.561$, $p=0.024$; $r=0.513$, $p=0.04$) as well as non-obese group ($r=0.687$, $p=0.05$; $r=0.578$, $p=0.024$).

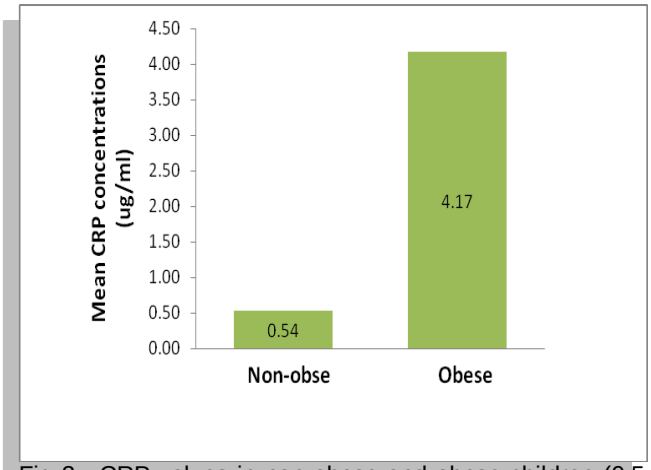

Fig 2: CRP values in non-obese and obese children (0.5-10 year old).

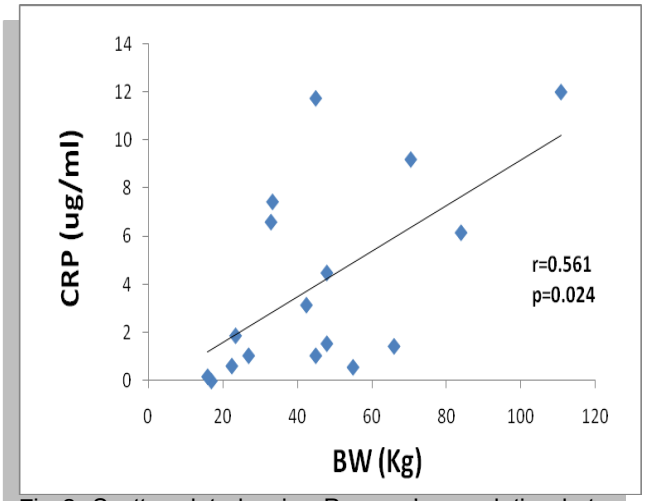

Fig 3: Scatter plot showing Pearson's correlation between CRP levels and BW in obese group.

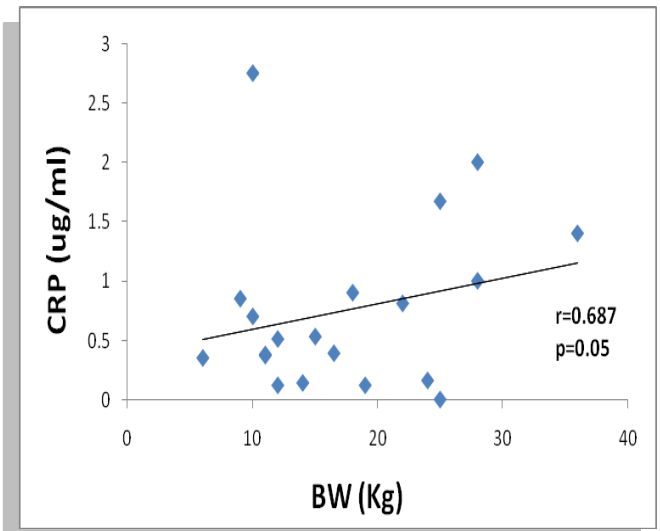

Fig 4: Scatter plot showing Pearson's correlation between CRP levels and BW in non-obese group.

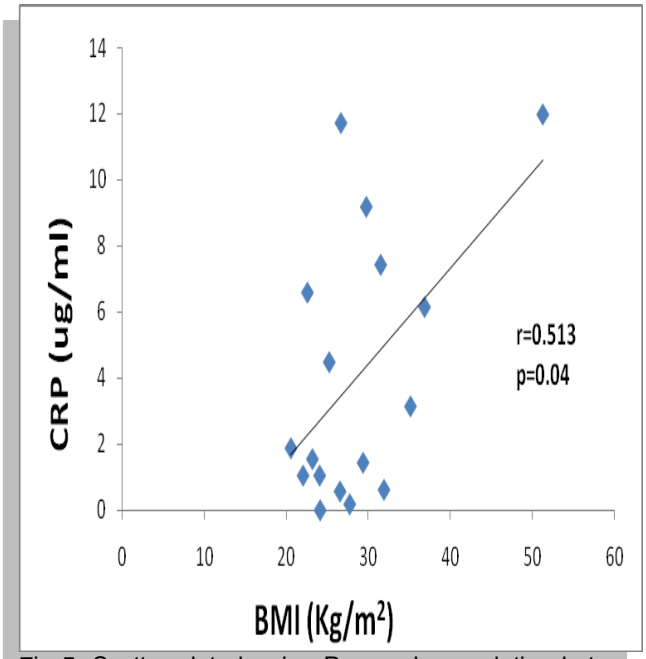

Fig 5: Scatter plot showing Pearson's correlation between CRP levels and BMI in obese group.

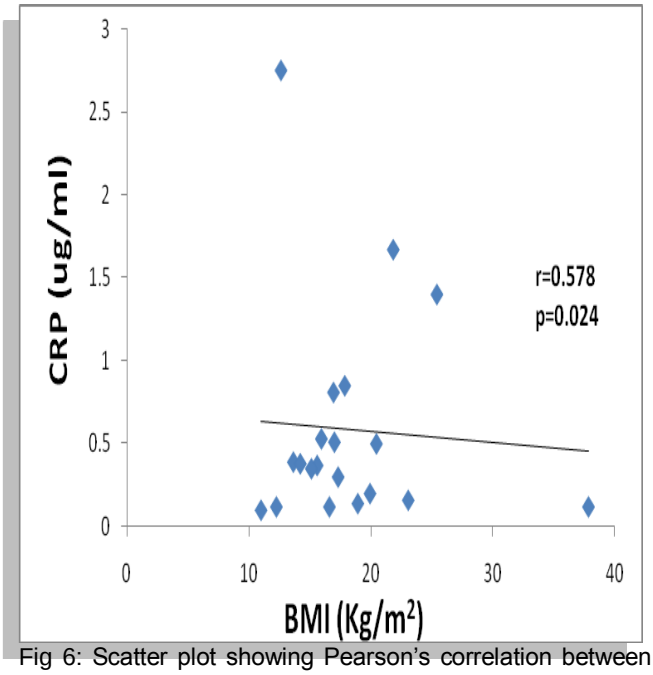

Fig 6: Scatter plot showing Pearson's correlation between CRP levels and BMI in non-obese group.

α-2macroglobulin*:* Mean α-2macroglobulin levels in the obese group (2.88±0.72) were significantly higher ($p<0.05$) from the non-obese (1.15 ±0.21). α-2macroglobulin levels ranged from 0.20-3.00 (0.75) µIU/ml in the lean subjects whereas 0.17-11.00 (1.98) µIU/ml in the obese children. In obese group, α-2macroglobulin was found significantly correlated with CRP levels ($r=0.604$; $p=0.017$), whereas no such correlation was observed in the lean group.

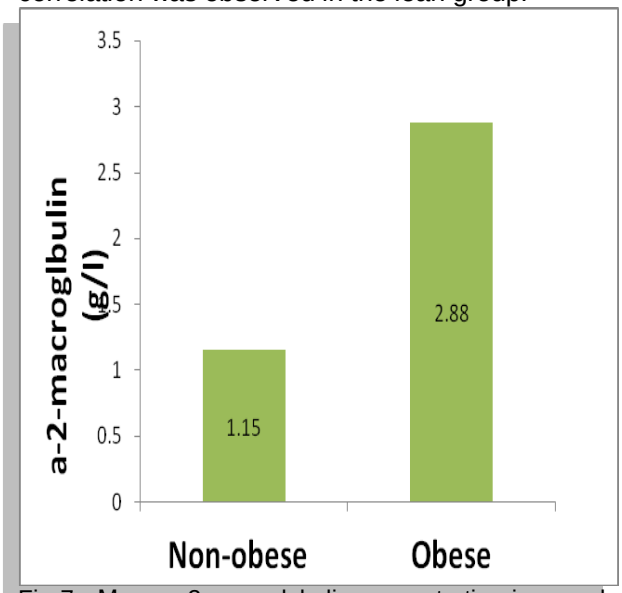

Fig 7: Mean α-2macroglobulin concentration in non-obese and obese children (0.5-10 year old).

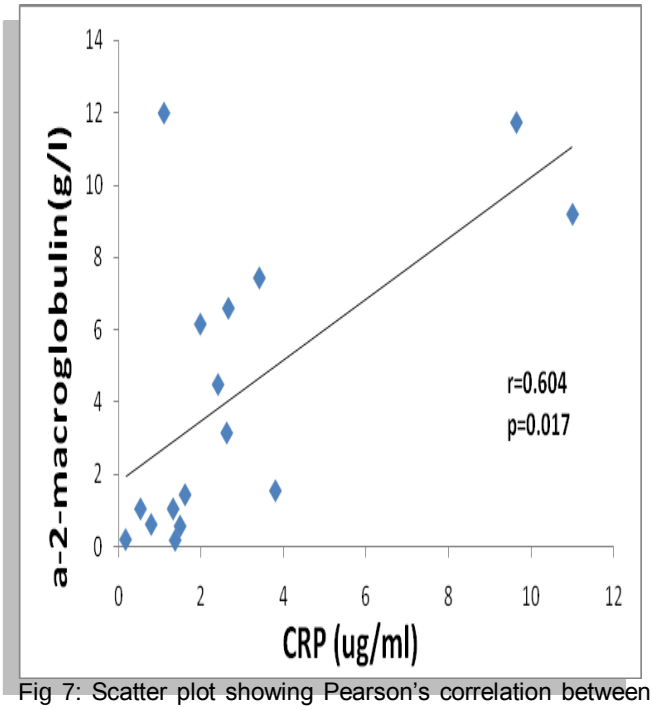

Fig 7: Scatter plot showing Pearson's correlation between α-2macroglobulin and CRP in obese group

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a. **Haptoglobin:** In the obese group, all (100%) subjects had increased hepatoglobin levels whereas, 50% of the non-obese had normal serum concentration of haptoglobin.

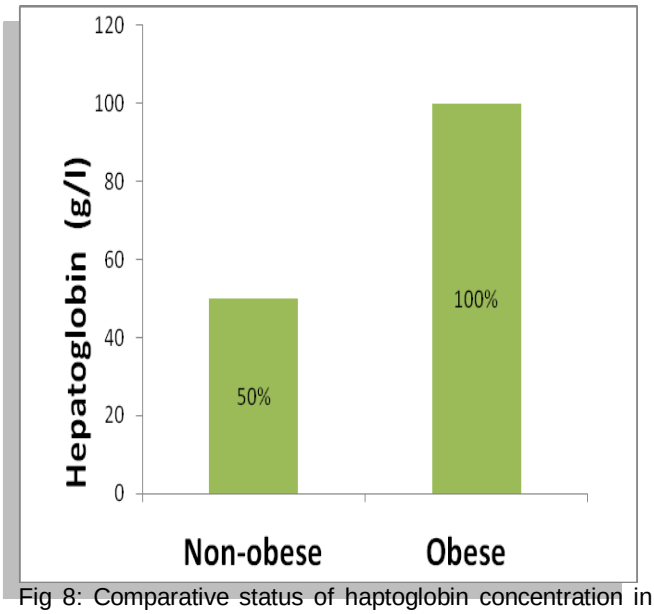

Fig 8: Comparative status of haptoglobin concentration in non-obese and obese children (0.5-10 year old).

DISCUSSION

This study was carried out to investigate and analyze pro-inflammatory acute phase markers in a group of rare cases of extreme obesity in children.

The association of obesity and low grade inflammation is strongly dependent on a degree of adiposity. Various studies have already associated increased levels of inflammatory markers with early stages of obesity. Only a few studies target the pathophysiology of childhood obesity.

The children recruited in the study comprised two groups based on their differences in body weight, height and BMI. Inclusion criteria included children with BMI percentile < of 50-80% for the lean (non obese) group and BMI percentile of >95% for the obese group. The body weight and height were directly correlated with age in both groups irrespective of increased adiposity in the obese children. Despite the above fact, BMI was markedly increased in the obese as compared to controls, although it was highly correlated with age within the group.

The serum concentration of CRP in all obese children was markedly higher than that of control group (4.07±0.98 vs. 0.53 ±0.16; $p<0.05$). Increased levels of CRP have also been reported in cases of common adult obesity and T2DM and are ascribed to the development of systemic inflammation[13,14].The elevated levels of CRP may be associated with increased cytokine secretions by the adipose tissue, where IL-6 and TNF-α are key players of the said process. We also found a positive correlation of CRP with body weight and BMI in both the group. However, further studies may provide us insight into whether increased CRP levels are a cause or a consequence of obesity in children.

Haptoglobulin levels have been associated with adipose mass[15]. It has also been evaluated for its role in inflammation in subjects with increased BMI. Moreover the increased synthesis of haptoglobulin has been thought to be a consequence of IL-6, secreted by adipocytes. All subjects in our study showed very high values of haptoglobulin; whereas only 50% of non obese had values in the normal range (0.3-3 g/l).The rest of the lean children may have some secondary cause that had resulted in an increase in haptoglobulin levels, as it has been reported as an inflammatory marker in altered homeostasis.

Mean serum concentration levels of A2M, another pro-inflammatory protein, were significantly high in obese when they were compared to age matched non-obese (2.88 ±0.72 vs. 1.15 ±0.21, $p<0.05$). A few previous studies have attempted to measure the levels of A2M in patients with obesity and metabolic syndrome [16]. Researchers could not derive equivocal conclusions as A2M levels were low in obese patients according to one study while they were higher than normal in another investigation. We also found a positive correlation between A2M and CRP ($r=0.604$; $p=0.017$) which may be indicative of the increase in primary cytokine leading to elevation of a secondary pro-inflammatory proteins.

The present study although does not clarify the exact picture of the causative factors and consequences of obesity associated inflammation, nevertheless highlights the role that immune-markers in the pathogenesis of the obesity and the accompanying insulin and leptin resistance.

CONCLUSION

The rate of obesity is increasing throughout the world. Environmental changes continue to occur in developed and developing countries creating a global pandemic with enormous implication of morbidity and mortality in the coming decades. On the basis of this study it is concluded that obese children undergo a pro inflammatory phase early in life. It is also seen that acute phase stress proteins are directly related with level of adiposity. It seems that we have a polygenetic risk to developing obesity and can only hope that the improved understanding of the causes and complex relationships of obesity will lead to better prevention and treatments.

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