



Role of inflammatory monocytes in adolescent metabolic syndrome



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Abstract

Metabolic syndrome (MetSyn) is a cluster of risk factors for cardiovascular disease and diabetes that affects 1 in 3 obese children. Inflammatory cytokines secreted from macrophages are thought to be partly responsible for the metabolic abnormalities associated with MetSyn. This study aimed to determine whether peripheral blood monocytes (macrophage precursors) from overweight individuals with MetSyn (Overwt-MetSyn) demonstrate enhanced activation of inflammatory signaling pathways when compared to metabolically normal overweight (Overwt-Healthy) or lean individuals. We conducted a cross sectional pilot study involving 24 adolescents (six boys and eighteen girls) recruited from the University of Massachusetts Boston and Worcester campuses. Six subjects were classified as Overwt-MetSyn using a modified definition proposed by the International Diabetes Federation. The Overwt-MetSyn group demonstrated an elevated expression of TLR2 and TLR4 in peripheral monocytes, and increased circulating levels of TNF α and IL6. Expression of TLR2 and TLR4 showed a positive correlation with circulating cytokines, and expression of TNF α correlated with waist circumference and BMI. Metabolic syndrome in adolescents is associated with increased activation of the TLR signaling pathway in monocytes. The knowledge gained from this study will advance our understanding of the contribution of monocytes to the pathophysiology of MetSyn.

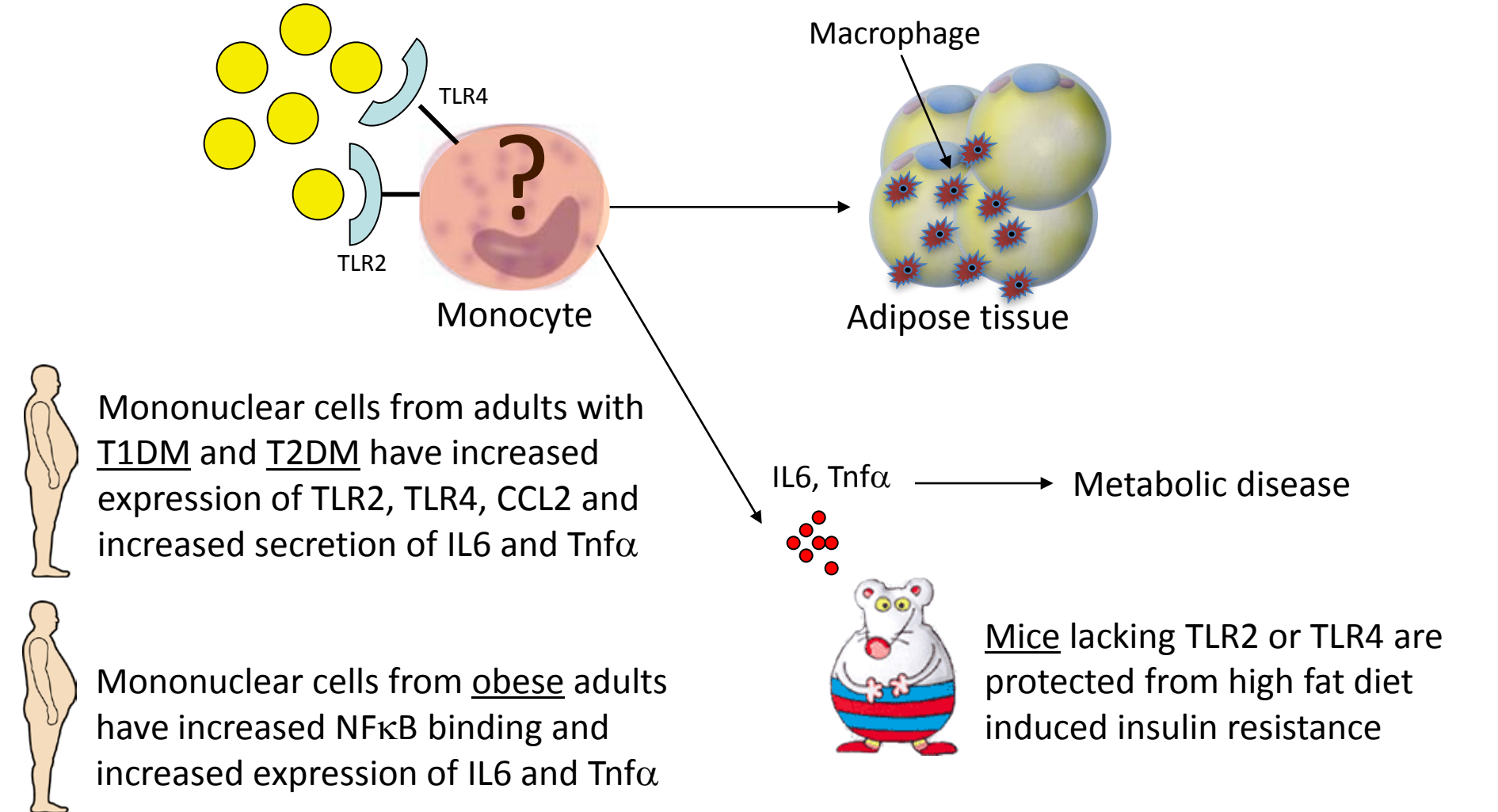
Background

•The recent epidemic of childhood obesity is placing countless children at risk for developing type 2 diabetes (T2DM), dyslipidemia, cardiovascular disease (CVD) and cancer

•Safe and effective pharmacological treatments are limited for obese children with metabolic disease

•Identification of the molecular and dietary triggers that link obesity with its associated co-morbidities is essential to recognize modifiable risk factors and target therapeutic interventions

•Currently, there is a paucity of data examining the expression and activity of TLR2 and TLR4 in obese adolescents



Devaraj S, et al., J Clin Endocrinol Metab. 2008; Bradshaw EM, et al., J Immunol. 2009; Cipolletta C, et al., Diabetes. 2005; Devaraj S, et al., Circulation. 2000; Devaraj S, et al., Free Radic Biol Med. 2000; Ghanim H, et al., Circulation. 2004; Caricilli AM. 2008. J Endo

FIGURE 1. TLR2 and TLR4 signaling is stimulated by nutritional fatty acids and high glucose both of which are often elevated in the obese state.

HYPOTHESES:

1. Monocytes from Overwt-MetSyn subjects will have increased gene expression of TLRs and cytokines when compared to Overwt-Healthy and Lean subjects
2. TLR and cytokine expression will show a positive correlation with anthropometric and serum markers of metabolic disease

Results

TABLE. Clinical and biochemical characteristics of the study participants

	Overwt MetSyn (n=6)	Overwt Healthy (n=9)	Lean (n=9)
Female, No. (%)	6 (100)	6 (67)	6 (67)
Age (yr)	16.5 (15.4-17.2)	16.8 (15.9-19.8)	16.6 (15.6-18)
Ethnic group, No. (%)			
African American	4 (67)	6 (67)	7 (78)
Caucasian	0	2 (22)	1 (11)
Hispanic	2 (33)	1 (11)	1 (11)
BMI (kg/m2)	39 (30-52)*	32 (26-40)*	21 (18-25)^
BMI %	98 (96-99)*	94 (85-99)*	50 (36-84)^
Waist circumference	117 (98-142)**	95 (80-112)*	74 (61-83)^
Systolic BP (mm Hg)	122 (102-139)	117 (108-141)	114 (104-129)
Diastolic BP (mm Hg)	75 (61-84)	71 (53-85)	73 (63-81)
White blood cell counts (k/uL)	8 (5-12)*	6 (4-9)	6 (4-7)
Monocytes (%)	8 (4-12)	9 (6-14)	8 (5-11)
Cholesterol (mg/dL)	119 (72-162)	113 (83-155)	109 (73-145)
Triglyceride (mg/dL)	61 (23-125)	48 (24-97)	51 (34-90)
HDL (mg/dL)	40 (32-47)	41 (24-56)	42 (29-56)
LDL (mg/dL)	67 (27-90)	62 (49-100)	57 (32-81)
CRP (pg/mL)	3.5 (1-11)	3.1 (1-16)	1 (1-1)
Fasting glucose (mg/dL)	98 (82-119)	89 (66-109)	95 (84-110)
Fasting insulin (uIU/mL)	13 (2-31)*	6 (2-14)	3 (2-6)^
HOMA-IR	2.9 (0.8-7.1)*	1.3 (0.3-3.3)	0.7 (0.4-1.4)^
Tnfa (pg/mL)	2.14 (0.75-4.66)**	0.86 (0-2.5)	0.95 (0.3-1.84)
IL6 (pg/mL)	2.75 (0.49-3.89)**	1.46 (0-2.74)*	0.61 (0-2.2)^

Data presented as mean (range)

* P < 0.05 compared with lean

** P < 0.05 compared with overweight healthy

^ P < 0.05 compared with all overweight (Overweight healthy and Metabolic syndrome)

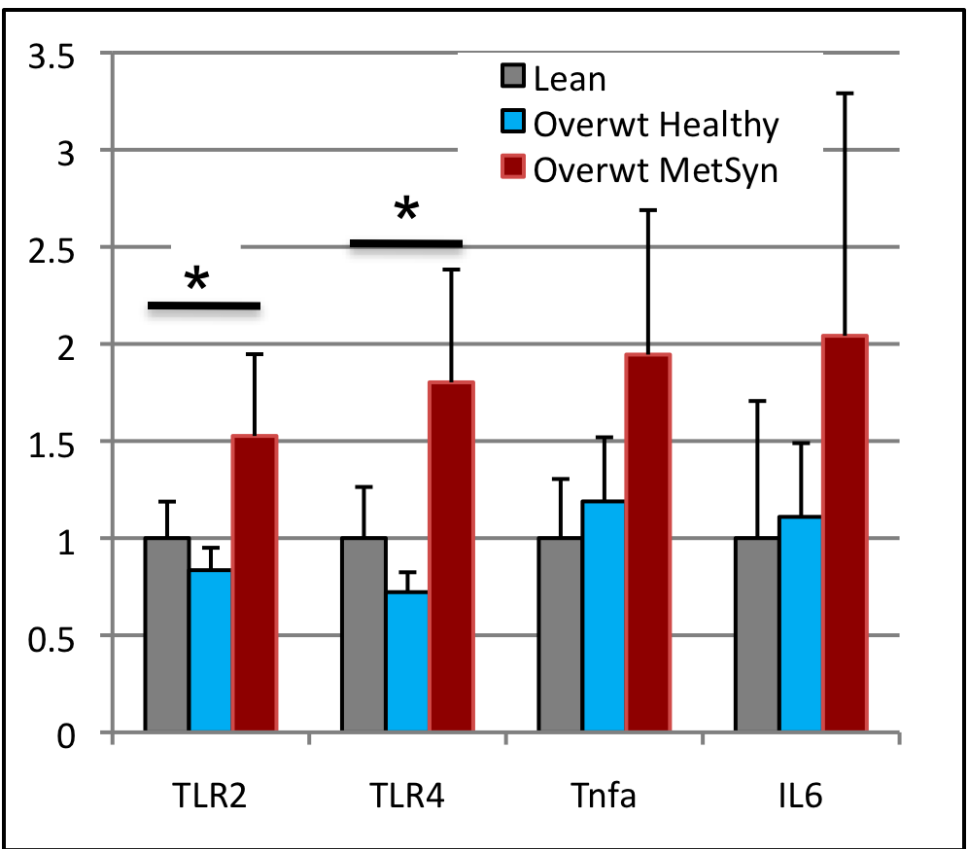


FIGURE 2. Monocytes from Overwt-MetSyn subjects display increased expression of inflammatory genes

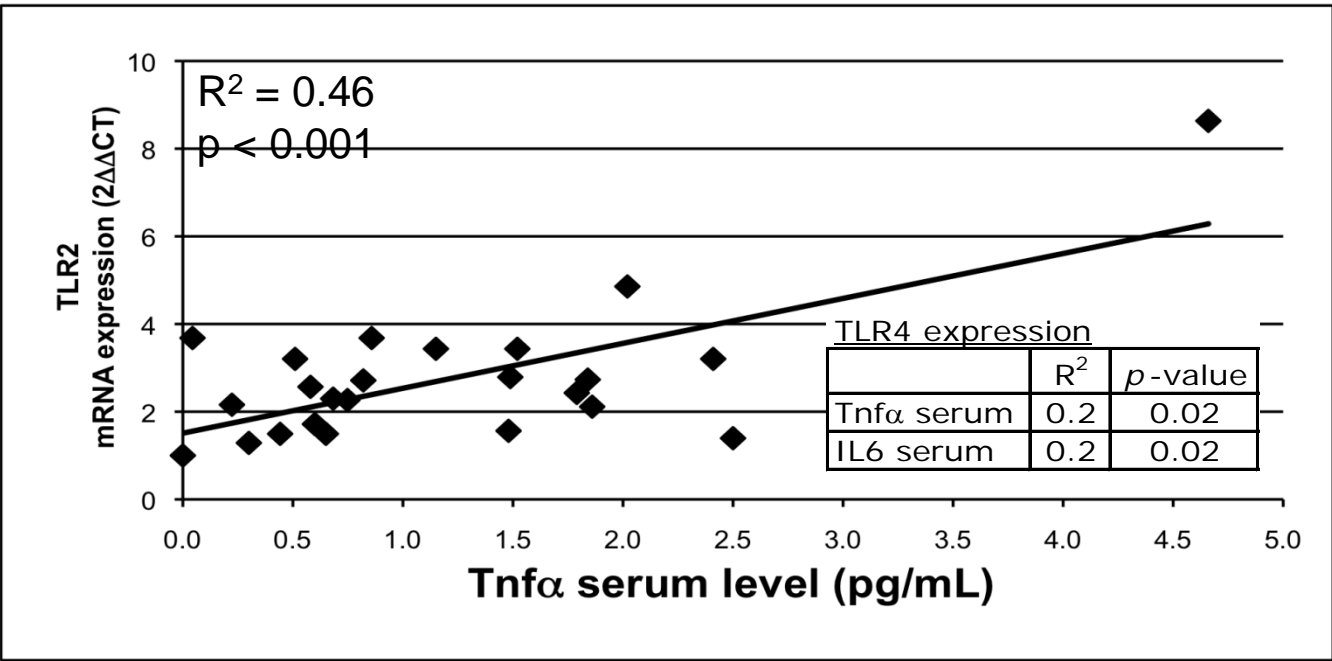


FIGURE 3. TLR expression in monocytes correlates with circulating cytokines

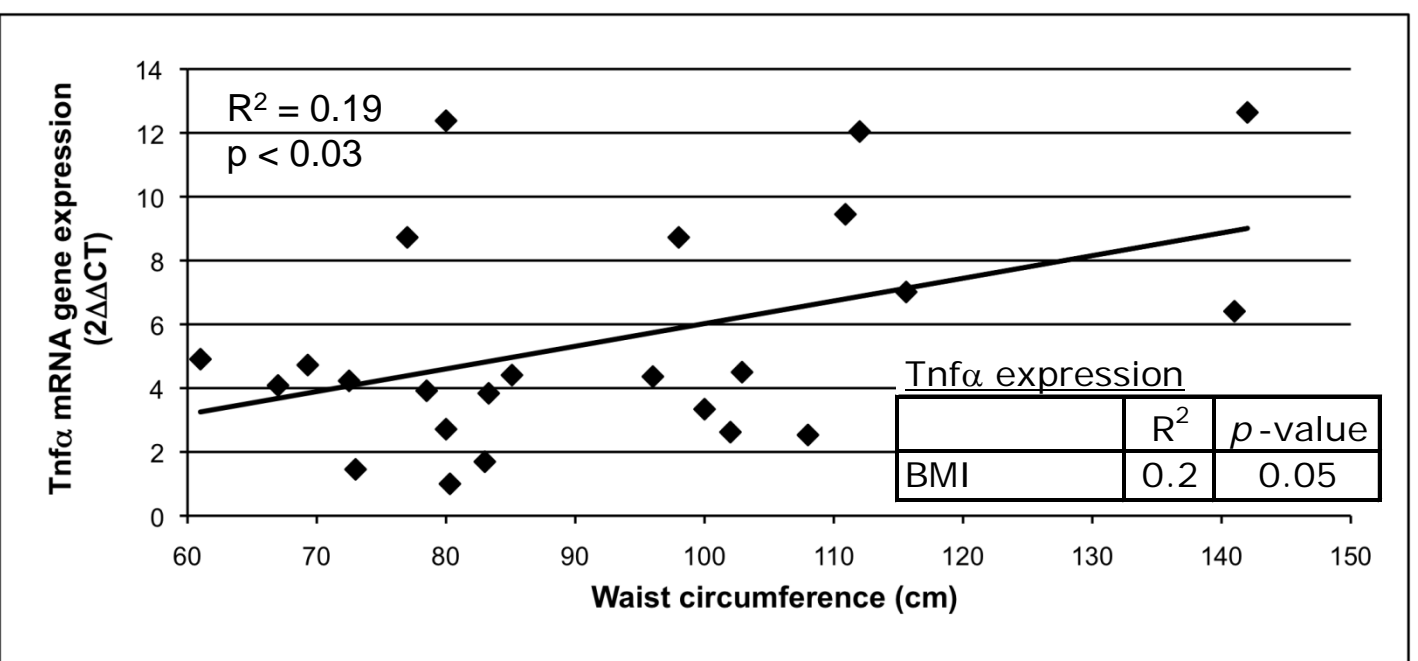


FIGURE 4. Cytokine expression in monocytes correlates with BMI and central obesity

Conclusions and Future Directions

1. Monocytes from Overwt-MetSyn subjects display increased gene expression of TLRs and cytokines
2. TLR expression shows a positive correlation with circulating cytokines; cytokine expression correlates with BMI and waist circumference

Ongoing research

1. Continue subjects recruitment
2. Assess TLR protein levels, surface markers
3. Measure secreted cytokines (Tnfa, IL6) from cultured monocytes at baseline and in response to TLR ligands and dietary lipids

Materials/Methods

Participants

The subjects included in this pilot study were recruited from the University of Massachusetts Boston and Worcester campuses. These consenting individuals provided a fasting blood sample and a limited history and physical examination were obtained. All subjects provided written informed consent before taking part in the study and the study was approved by the University of Massachusetts Institutional Review Board. Subjects were classified as Overwt-MetSyn using a modified definition proposed by the Adult Treatment Panel III as ≥ 3 of the following: (1) fasting triglycerides > 100 mg/dL; (2) HDL < 50 mg/dL (except in boys aged 15 to 19 years, in whom the cutpoint was < 40 mg/dL); (3) fasting glucose ≥ 100 mg/dL; (4) waist circumference >75th percentile for age and gender; and (5) systolic blood pressure >90th percentile for gender, age, and height. Exclusion criteria for study participation include the following: (a) medical history of type 1 or type 2 diabetes, (b) any acute or chronic inflammatory disease (i.e. rheumatoid arthritis, ulcerative colitis), (c) cardiovascular or peripheral artery disease, (d) thyroid dysfunction, (e) Cushing's disease or hypercortisolism, (f) pregnancy (by self-report), (g) medications that may affect outcome parameters (metformin, lipid lowering medication, antihyperglycemic agents, oral steroids), (h) genetic disease resulting in obesity, (i) presence of an eating disorder.

Blood sample collection and monocyte isolation

Blood samples were obtained via venipuncture after an 8 hour overnight fast. Venous blood (15 mL total) was collected in two Vacutainer™ Cell Preparation Tubes (Becton Dickinson, Rutherford, NJ) with Na Citrate which are intended for the collection of whole blood and the separation of mononuclear cells using a FICOLL™ Hypaque™ solution. An additional 5 mL was collected in an EDTA tube (Vacutainer™, Becton Dickinson) for whole blood hematology analysis. Blood mononuclear cells were isolated from the Vacutainer™ Cell Preparation Tubes via centrifugation. Untouched primary monocytes were further isolated from the mononuclear fraction using a negative selection kit (Miltenyi Biotec, Germany).

Quantitative PCR and serum inflammatory markers

RNA was isolated from monocytes according to the QIAGEN MiniPrep protocol. cDNA from total RNA was synthesized using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories). Quantitative real-time PCR was used to quantify expression of TLR2, TLR4 and 2 inflammatory cytokines using SybrGreen assays according to manufacturer's instructions (Bio-Rad Laboratories). Expression of specific mRNAs was quantified in duplicate samples on an iCycler IQ Real-Time PCR detection system (Bio-Rad Laboratories) using the $\Delta\Delta C_T$ method with normalization to cycle threshold measurements for GAPDH.

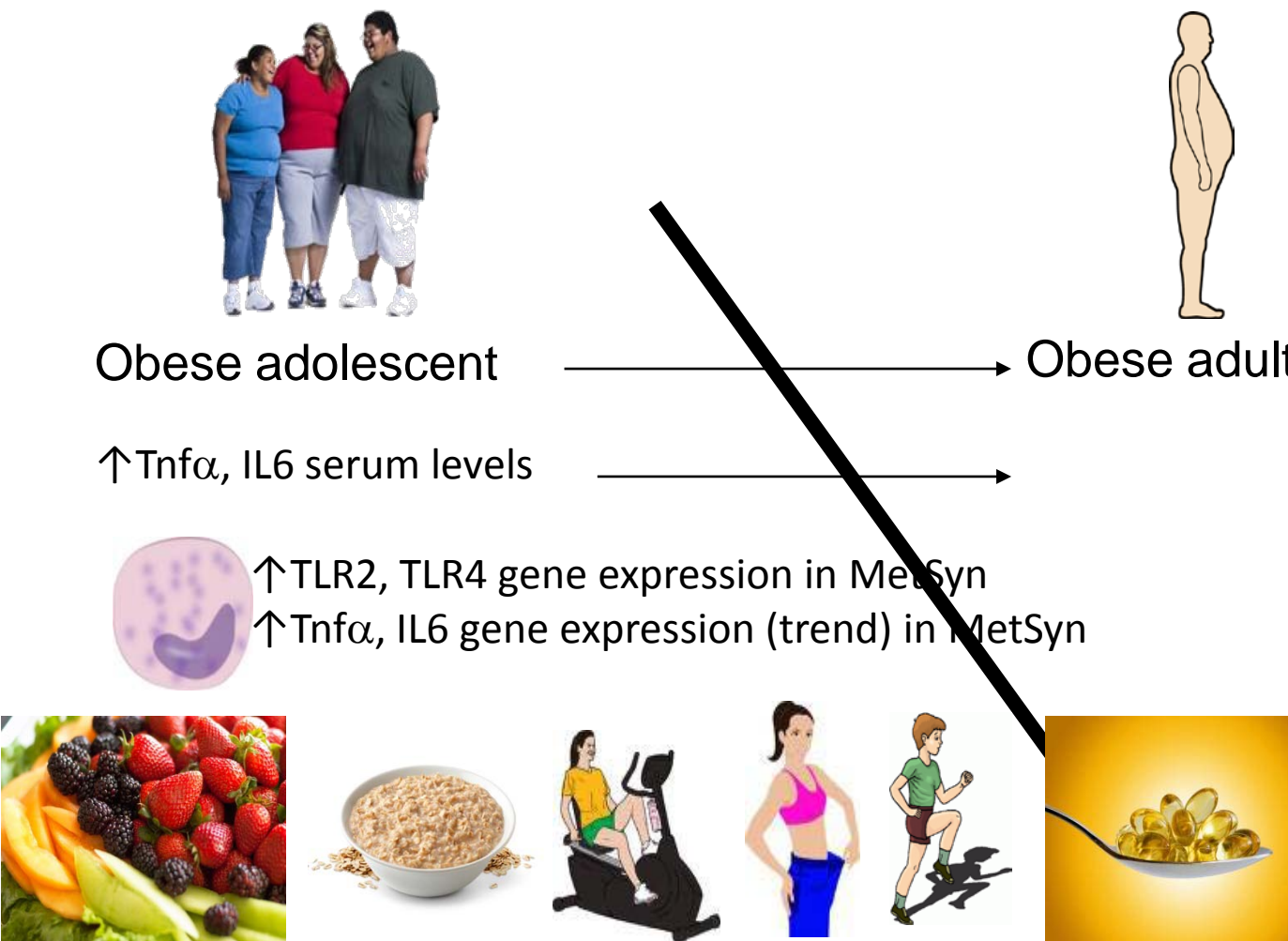
Serum levels of inflammatory cytokines of interest (TNF α and IL6) were measured in the initial fasting blood samples using ultra-sensitive ELISA kits (R&D Systems).

Data Management

In our primary analyses, data from Overwt-MetSyn subjects and Overwt-Healthy subjects was compared to determine the effect of MetSyn on these outcome measures. In a secondary analysis, data from Overwt-Healthy and Lean subjects was compared to determine the effect of obesity on these outcome measures. Correlations were assessed using Pearson's coefficient. For all of the analyses, the results were considered significant at P ≤ 0.05 .

Acknowledgments

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Questions

1. Impact of dietary changes on monocyte inflammation?
2. Improvement in monocyte inflammation with weight loss and/or exercise?
3. Reversal of monocyte inflammation with pharmacotherapy or nutritional supplements?