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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 TNFα and IL6. Expression of TLR2 and TLR4 showed a positive correlation with circulating cytokines, and expression of TLRs 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

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

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 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 2 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 > 75 percentile for age and gender; and (5) systolic blood pressure > 90 percentile for gender, age, and height.

Blood sample collection and monocyte isolation

Blood samples were obtained via venipuncture after an 8 hour overnight fast. Various blood (15 mL total) was collected in two VacutainerTM 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 FICOLLTM HypaqueTM solution. An additional 5 mL was collected in an EDTA Vacutainer® tube (Vacutainer®, Becton Dickinson) for whole blood hematological analysis. Mononuclear-cells were isolated from the Vacutainer® Cell Preparation Tubes by centrifugation. Untouched primary monocytes were further isolated from the mononuclear fraction using a negative selection kit (Milteny Biotec, Germany).

Quantitative PCR and serum inflammatory markers

RNA was isolated using the TRIzol reagent according to the manufacturer抯 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抯 instructions (Bio-Rad Laboratories). Expression of specific mRNAs was quantified in duplicate samples on an iCyicer IQ Real-Time PCR detection system (Bio-Rad Laboratories) using the ΔΔCt method with normalization to cycle threshold measurements for GAPDH.

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抯 coefficient. For all of the analyses, the results were considered significant at $P < 0.05$.

Acknowledgments

This study was partially supported by a grant from the Life Sciences Momentum Fund (OHL,ULW, LHT), University of Massachusetts Medical Center and supported by the Diabetes Endocrinology Research Center grant DK32520

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

Questions

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