Title
Effect of Diet Intervention on Inflammation-Related Gene Expression in CD14+ Circulating Monocytes from Metabolic Syndrome Patients

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Chronic low-grade inflammation is associated with metabolic syndrome and insulin resistance, which is the primary defect leading to type 2 diabetes mellitus. In humans, dietary interventions have been shown to increase insulin sensitivity, with one of the hypothesized mechanisms being reduced activation of pathways that promote chronic inflammation. The objective of this study was to analyze pro-inflammatory gene expression in CD14+ circulating monocytes from metabolic syndrome subjects before, during, and after two diet interventions. The study was a randomized, single-blind, controlled trial of a 12-week long, 1500 calorie diet. The active diet included low glycemic index bread products, EPA/DHA fish oil capsules (2.4g EPA+DHA/day), and delphinidin polyphenol capsules (300 mg, 10,000 ORAC units/day). The placebo diet included market variety bread products, corn oil capsules, and corn starch capsules. We hypothesized that the active dietary components would act synergistically to significantly improve the inflammatory gene expression profile of the subjects as compared to placebo diet. Baseline, midpoint, and endpoint mRNA expression profiles from monocyte RNA samples were assessed for 30 obese subjects. Post-hoc analysis of real-time quantitative polymerase chain reaction (RT-qPCR) results to measure the expression levels of twelve inflammatory marker genes of interest revealed significant reduction in toll-like receptor 2 (TLR2), CD11c, and tumor necrosis factor alpha (TNFα) expression in circulating monocytes of active group as compared to the placebo group, P<.05. This study was part of a parent clinical trial showing that average fasting insulin and HOMA-IR improved significantly in the active group but not the placebo group. This monocyte gene expression data will contribute to painting the picture of how the composition of a low calorie diet modulates inflammation and insulin sensitivity.
EFFECT OF DIET INTERVENTION ON INFLAMMATION-RELATED GENE EXPRESSION IN CIRCULATING MONOCYTES FROM METABOLIC SYM DROME PATIENTS

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BACKGROUND

• Metabolic syndrome is a state characterized by insulin resistance and other metabolic parameters, including increased body mass index (BMI), hypertension, dyslipidemia, and increased waist circumference, which confer a high risk for developing type 2 diabetes (T2DM).
• Chronic low-grade inflammation is associated with metabolic syndrome and insulin resistance, which is the primary defect leading to T2DM.
• Many genetic studies have reinforced this, showing that when genes involved in inflammatory pathways are knocked out, mice on high-fat diets are protected against insulin resistance.
• In humans, lifestyle-based interventions targeting inflammation have been linked to increased insulin sensitivity. However, there is a deficit in studies analyzing the precise effect of a multi-component anti-inflammatory diet on cytokine expression.
• It has been shown that low-glycemic index foods have a protective effect on hyperglycemia-associated oxidative damage, improve insulin sensitivity, and improve metabolic parameters.
• Ellagitannins and other flavonoids have been shown to reduce chronic inflammation and improve insulin sensitivity.
• Anthocyanin polyphenols, under which delphinidins are classified, have been found to have anti-diabetic effects due to their ability to combat the oxidative stress associated with obesity.

MATERIALS AND METHODS

Subjects: CD14+ white blood cells were isolated from 31 obese subjects (average BMI 35.4 ±0.6 kg/m²) between the ages of 21 and 55. Enrollees also met the criteria for having metabolic syndrome, defined as waist circumference >102 cm for men, >88 cm for women, and any two of the following: TG>150 mg/dL, HDL<40 mg/dL for men or <50 mg/dL for women, fasting blood glucose 100-125 mg/dL, acanthosis nigricans, or blood pressure ≥130/85.

Diet: The intervention was a randomized controlled trial of a 12-week long, 1500 calorie diet. The active diet included reduced glycemic index bread products, EPA/DHA fish oil and delphinidin polyphenol capsules. The placebo diet included market variety bread products, corn oil and corn starch capsules. Blood samples were collected at weeks 0 (baseline), 6 (midpoint), and 12 (endpoint) from which monocytes were isolated.

RT-qPCR: RNA from monocyte samples was extracted using Invitrogen TRIZol reagent according to the manufacturer’s protocol. Libraries of CDNA were constructed using Invitrogen cDNA synthesis kit according to the manufacturer’s protocol. 5 μg CDNA were used as the template for a 10 μl RT-qPCR reaction for each sample. Primer sequences for TLR2, TLR4, NFKB, TNFα, TNFαF5′F1, IL-1β, IL-12, IL-6, CDX2, CD11c, INOS and OPN were designed via NCBI PrimerBlast. Detection of 18S cDNA was performed on a plate to plate variance. Data was reported as “normalized expression”: the inverse of threshold cycle for the gene of interest normalized to threshold cycle for the 18S target.

Statistical Analysis: A repeated measures MANCOVA was conducted to examine the effects of two diet groups on multiple gene expression dependent variables (TLR2, TLR4, NFKB, TNFα, TNFαF5′F1, IL-1β, IL-12, IL-6, CDX2, CD11c, INOS and OPN) pre-, mid-, and post-12 week intervention. A p-value cutoff of 0.05 was used for significance.

RESULTS

MANCOVA using paired, multiple time points revealed significant difference in gene expression change by group for CD11c, F (1, 15) = 12.484, p = 0.003, and TNFα, F (1, 15) = 7.805, p = 0.013. Baseline expression of these two genes was not significantly different between groups.

SUMMARY

• At the end of the diet intervention, the change in CD11c and TNFα expression was less in the active group compared to the placebo group.
• Controlling for the baseline expression difference and functional interaction between NFKB and TLR2 revealed significantly reduced expression of CD11c, TLR2, TNFα at the midpoint and significantly reduced expression of IL-6 and TNFα at the endpoint.
• In conclusion, these data suggest that variations in composition of a low calorie diet can differentially influence monocyte inflammatory gene expression. These findings parallel beneficial metabolic outcomes for the active diet not observed with the placebo diet that were independent of weight loss and improved lipid panel.

REFERENCES


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