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Article

Transcriptomic Differences Between Monozygotic Adolescent Twins Discordant For Metabolic Syndrome Following Weight Loss: A Case Study

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Abstract

This case reports peripheral blood mononuclear cell (PBMC) transcriptomic changes in a pair of male monozygotic pediatric twins with metabolic syndrome (MetS) undertaking assisted weight loss. These 14-year-old boys presented with similar baseline biochemistry and body composition. After a 16-week weight-loss intervention, percent body weight loss was similar (Twin A 12%, and Twin B 13%). MetS resolved in Twin A but Twin B maintained elevated triglycerides after weight loss. Analysis of the PBMC transcriptome before and after weight loss revealed very different changes in gene expression including differences in the direction of expression of genes related to immune cell activation. 48.7% of genes that were downregulated in Twin A were upregulated in Twin B. This case highlights a novel approach to report the influence of chronic low-grade inflammation and metabolic dysfunction on the PBMC transcriptome. It explores whether expression of genes related to immune functions may underlie the differences in response to weight loss or whether transcriptomic alterations in immune cells may precede more traditional biomarkers of chronic pro-inflammation. These monozygotic twins present an example of divergence of phenotypic outcomes despite identical genetic background and similar treatment response.

Keywords: Pediatric obesity; gene expression; metabolic syndrome

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This case reports on a pair of adolescent male monozygotic twins who presented to a tertiary hospital with metabolic syndrome and obesity, seeking weight loss treatment. Upon 16 weeks of a weight-loss intervention, metabolic syndrome resolved in one twin only, and this was accompanied with differential changes in the peripheral blood mononuclear cell (PBMC) transcriptome between twins. Ethical approval for this study was provided by Monash Health HREC (RES-17-0000-519X), and the family provided additional written consent for this case study publication.

Case Presentation

Medical records indicated that the twins were born at 34 weeks' gestation using forceps to assist delivery. Zygosity was self-reported as monozygotic. Twin A had a birth weight of 2.005 kg and Twin B had a birth weight of 2.44 kg. Twin A had an uncomplicated neonatal course, but Twin B required CPAP for respiratory distress syndrome and polycythemia requiring a partial exchange transfusion. They were raised together by their genetic parents in the same home environment. Twin A has a history of learning disabilities and visual impairment in the context of macrocephaly and white matter changes of antenatal origin on brain MRI. By the age of 10, both twins had developed significant obesity with concurrent metabolic dysregulation that met the criteria for metabolic syndrome (MetS) as defined in adolescents by Zimmet et al. (2007). Both twins presented for treatment at a tertiary teaching hospital at age 14, advanced in puberty. They underwent a 16-week dietary protocol for weight loss in a dietitian-led weight management program. Measurements were taken at baseline and week 16 after an overnight fast. Both twins achieved >10% body mass loss (Twin A: 12% reduction; Twin B: 13% reduction) with concurrent reductions in waist circumference, fat mass and blood pressure (Table 1). There were no notable differences between twins in the reduction of fat mass (kg) or waist-to-height ratio (WtHR), but Twin B experienced a slightly greater reduction in waist circumference (A: −13.7 cm vs. B: −16.4 cm), and grew in stature slightly more than his brother (A: +0.55 cm vs. B: +1.8 cm) during the 16 week intervention. The reduction in fasting triglycerides was greater in Twin A (−2.0mmol/L) than

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Table 1. Anthropometric, body composition and biochemistry outcomes at baseline and week 16.

	Baseline		Week 16	
	Twin A	Twin B	Twin A	Twin B
Birth weight (kg)	2.01	2.44		
Weight (kg)	112.3	107.7	98.2	93.3
Height (cm)	176.9	178.25	177.45	180.05
BMI (kg/m^2)	35.85	33.99	31.34	28.80
BMI Z-score (percentile)	2.49 (99)	2.37 (99)	2.17 (98)	1.94 (97)
Waist circumference (cm)	107.23	110.37	93.53	93.9
WtHR	0.61	0.62	0.53	0.52
Fat mass (kg)*	47.32	45.79	33.43	32.46
Fat free mass (kg)*	63.43	60.56	63.65	59.21
Percentage fat mass loss (%)			−29.29	−29.11
Blood pressure (Systolic/Diastolic, mmHg)	120.3/72.3	114.3/72.3	119.0/66.3	111.7/69.7
Fasting glucose (mmol/L)	4.4	4.6	4.6	5.4
Fasting insulin (mIU/L)	17.9	18.9	8.0	12.3
Fasting triglycerides (mmol/L)	3.1	3.2	1.1	2.7
Fasting total cholesterol (mmol/L)	5.3	5.3	3.9	4.1
HDL (mmol/L)	0.9	1	0.9	0.9
LDL (mmol/L)	3	2.9	2.5	2
CRP (mg/L)	3.7	5	6	1.7
Metabolic syndrome (Y/N)**	Y	Y	N	Y

Note: BMI, body mass index; WtHR, waist to height ratio; CRP, C reactive protein. *Fat mass and fat free mass as measured by dual X-ray absorptiometry. **Metabolic syndrome defined using the criteria set out by the International Diabetes Federation (Zimmet et al., 2007).

Twin B (−0.5mmol/L), such that Twin A (but not Twin B) no longer met the adolescent criteria for MetS.

RNA Extraction and Analysis

RNA was isolated from PBMCs collected at baseline and after weight loss at week 16. RNA integrity (RIN) was measured using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, California, United States). Samples were advanced to RNA sequencing (Illumina NextSeq 500, Illumina, San Diego, California, United States) with a RIN above 8; all twin samples had a RIN above 8. For immune cell subsets within PBMC samples, enrichment scores were calculated using SPEC (version 0.5.0; Bolen et al., 2011) whereby the twins' gene expression signatures were correlated with the default package gene signatures for each cell type (T cells, myeloid cells, monocytes and lymphocytes).

Fastq files of raw sequence reads were aligned to the human genome (GRCh38) using the STAR aligner and read counts generated using the featureCounts package as part of the 'RNAsiq' pipeline (Tsyganov et al., 2018) developed by the Monash Bioinformatics Platform (Monash University, Melbourne, Australia). Only genes with a mean count >10 were included in the downstream analysis. Due to the case study design, genes were determined as differentially expressed with a log-fold change (logFC) <-0.26 or >0.26 (Muñoz García et al., 2019), a cut-off used in nutrition research to define differentially expressed genes, since standard statistical testing could not be performed in this case ($N = 2$). To explore the biological processes that differentially expressed genes were associated with, these genes were mapped to gene ontologies (GO) using the ClueGO app via the CluePedia plugin for Cytoscape (version 3.8.2). GO terms were considered enriched with an adjusted p value < .05 after Bonferroni stepped-down correction for false discovery rate (Dunn, 1961), and at least five included genes were mapped to the GO term. Terms were grouped into networks using a connectivity kappa threshold of 0.4.

Table 2. Correlation matrix between twin gene expression signatures and SPEC PBMC subset gene signatures before and after the weight loss intervention.

	Baseline		Week 16	
	Twin A	Twin B	Twin A	Twin B
T cells	0.74	0.60	0.67	0.64
Myeloid cells	0.26	0.19	0.35	0.27
Lymphocytes	0.56	0.39	0.46	0.57
Monocytes	0.52	0.43	−0.42	0.56

Results

Peripheral Blood Mononuclear Cell Subpopulations

SPEC analysis demonstrated that cell populations were comparable between twins with both twin gene signatures most strongly correlated with the T-cell gene signature both before and after the intervention (Table 2).

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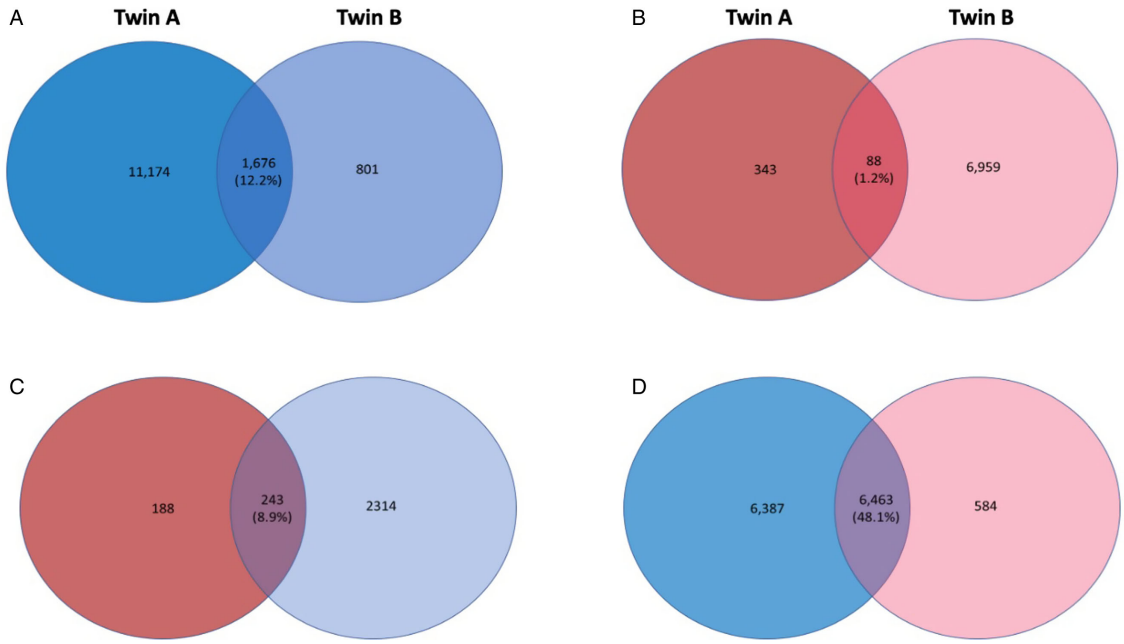

Fig. 1. Venn diagrams of gene expression changes following a 16-week lifestyle intervention in a set of monozygotic twins. A. Transcripts that were downregulated following the intervention (12.2% overlap between twins). B. Transcripts that were upregulated following the intervention (1.2% overlap between twins). C. Transcripts that were upregulated following the intervention in Twin A and downregulated in Twin B (8.9% overlap between twins). D. Transcripts that were downregulated following the intervention in Twin A and upregulated following the intervention in Twin B (48.1% overlap between twins).

Baseline Gene Expression

A mean count of >10 was detected in a total of 15,071 genes across all samples. At baseline, 4369 genes (28%) were found to have a logFC of either >0.26 (1179 genes) or <−0.26 (3189 genes) between twins. Gene ontology analysis found 64 GO terms associated with genes that were upregulated, and 222 GO terms associated with genes that were downregulated in Twin A compared to Twin B at baseline.

Gene Expression Following the Intervention

Following the intervention, 13,281 genes were differentially expressed in Twin A compared to baseline, and in Twin B, 9604 genes were differentially expressed. Very few genes (12.2% and 1.2%) were differentially expressed in the same direction following weight loss in the twins (Figure 1). In contrast, following weight loss, 6463 transcripts (48.1%) were downregulated in Twin A and upregulated in Twin B (Figure 2). Due to the large number, only those genes with a logFC <-0.58 or >0.58 following weight loss were used in the downstream analysis (2280 genes), which represents a more conservative cut-off commonly used in medical research (Rapaport et al., 2013). Ninety GO terms were enriched (adjusted p value < .05) between the 2280 genes inversely regulated following weight loss. Nine of the 10 top enriched terms were related to immune cell activation, with four of these terms relating specifically to neutrophil functions (Table 3). Nine of the top ten terms were also enriched between genes that were upregulated in Twin A at baseline compared to Twin B.

The majority of the top terms related to the broader ontologies of 'granulocyte activation' and 'leukocyte degranulation'; the interaction network between these terms is shown in Figure 1. These terms were also enriched between genes with lower expression levels in Twin B than Twin A at baseline.

Discussion

This case report presents the differential changes in the PBMC transcriptome between adolescent monozygotic male twins before and following a 16-week weight loss intervention. Both twins achieved a >10% body weight reduction (Twin A: 12%, Twin B: 14%) after a 16-week dietitian-led weight management program, with the majority comprised of fat mass loss. This level of body composition change would be anticipated to result in optimal metabolic changes and resolve MetS (Look AHEAD Research Group, 2014). However, while changes in some markers of MetS improved in Twin B, and indeed showed larger reductions than Twin A, the criteria for MetS were still met in Twin B following the intervention due to continued elevated triglycerides. These shifts in metabolic markers following weight loss were accompanied by differences in gene expression changes, with nearly half (48%) of the genes that decreased in expression in Twin A, increased in Twin B. Genes related to immune function (leukocyte degranulation and granulocyte activation) were upregulated at baseline but downregulated following the intervention in Twin A compared to Twin B, suggesting Twin A had a greater capacity for change in expression of genes related to immune functions than Twin B. The baseline discordance observed in gene expression related to immune function between the twins may underlie the differences in gene expression following the intervention.

Differences in whole blood and PBMC gene expression levels have been previously identified between people with metabolically healthy and metabolically unhealthy obesity, particularly for genes related to lipid metabolism and some immune functions (*TRIM11, ADAMTSL2*; Plaza-Florido et al., 2021), suggesting that differences in gene expression may underlie different metabolic phenotypes and transcriptomic differences could, in this case, precede differences in classical pro-inflammatory markers. A recent review into differences in PBMC gene expression between responders to a weight-loss intervention demonstrated

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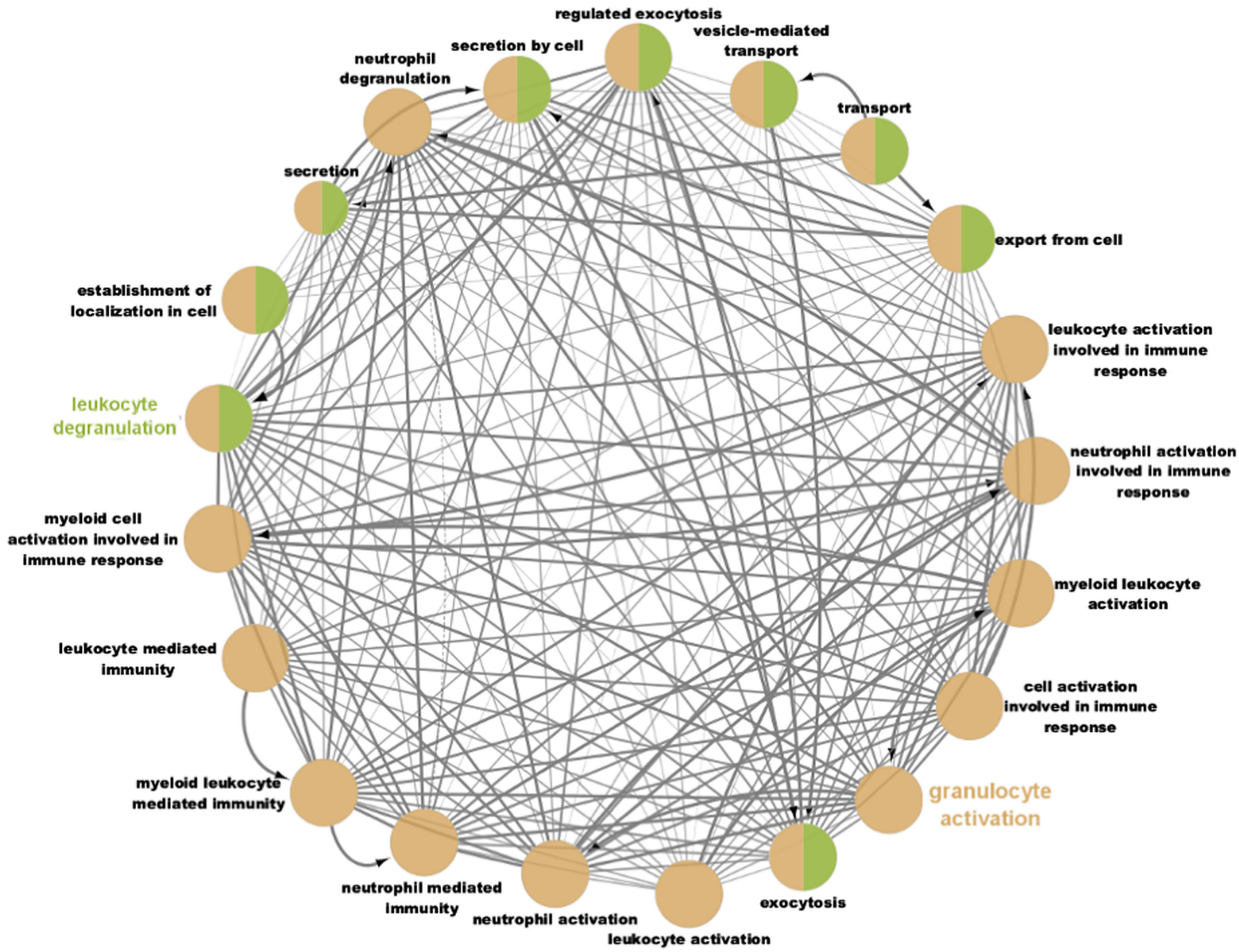

Fig. 2. Network connectivity map of gene ontology terms enriched for the genes downregulated in twin A and upregulated in twin B following weight loss (log-fold change > −.58 or < −0.58, 2280 genes). Terms were considered enriched with an adjusted FDR < .05 and grouped into networks with a connectivity kappa threshold of 0.4. These networks were broadly characterised as leukocyte degranulation and granulocyte activation.

consistent changes in toll-like receptor signalling between high responders to a weight loss intervention (Day et al., 2021), which belongs to the same broader gene ontological process of 'activation of immune response' as leukocyte and neutrophil activation, which were observed in the current study (Figure 2). A study into PBMC gene expression of African American males with obesity observed that neutrophil specific genes were upregulated in obesity, suggesting a role of neutrophils in obesity and its treatment (Xu et al., 2015).

Both genomewide association studies and twin studies have been used to characterize the heritability of body weight and body shape (Locke et al., 2015; Min et al., 2013; Thorleifsson et al., 2009). Genomewide association studies have suggested that less than 1 −3% of BMI variation can be explained by differences in genotype (Locke et al., 2015; Thorleifsson et al., 2009). Despite this, a systematic review of twin studies has suggested moderate heritability of weight at birth, which increases to a maximum heritability of 79% at around 20 years of age before waning again into adulthood (Min et al., 2013); suggesting that the genetic influence of body weight wanes with increasing age and that environmental impacts may accumulate over time before reaching a critical threshold to impact phenotype. Interestingly, this places the twins reported here close to their peak age of body weight heritability. Indeed, body weight at baseline and follow-up were similar between twins, despite differences in gene expression responses at both baseline and in response to the dietary intervention. The birth weights of the twins described here were borderline discordant (17.8% difference), compared to the criteria (≥18% difference) for assessing birthweight-associated adverse outcomes (Breathnach et al., 2011). In several large cohort and twin studies, low birth weight has been associated with obesity and lower lean mass in adulthood, independent of genetic and maternal influences (Hertfordshire Study, 2005; Labayen et al., 2008; Loos et al., 2001). Low birth weight is an indicator of in-utero stress, which can be caused by multiple factors, including undernutrition (Mayer & Joseph, 2013) and multiple pregnancy (Sankilampi et al., 2013). Multiple pregnancies may be more susceptible to fetal undernutrition due to competition for maternal and placental supply, and this competition may lead to differences in birth weight that can impact upon body weight and adiposity later in life (Fox et al., 2011). In the twins described here, the twin with a lower birth weight presented at baseline with a higher BMI, waist circumference, and percentage body fat, although there were no differences in the criteria for MetS.

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Table 3. The 10 most enriched gene ontology terms for the genes oppositely regulated between the twins following weight loss. All top ten terms were significantly enriched after Bonferroni adjusted for multiple testing (adj.*p* value < .01).

GO_term	Name
GO:0036230	Granulocyte activation
GO:0042119	Neutrophil activation
GO:0002274	Myeloid leukocyte activation
GO:0002283	Neutrophil activation involved in immune response
GO:0043312	Neutrophil degranulation
GO:0043299	Leukocyte degranulation
GO:0002275	Myeloid cell activation involved in immune response
GO:0002446	Neutrophil mediated immunity
GO:0006793	Phosphorus metabolic process

The contrasting response of the PBMC transcriptome following similar weight loss in these twins is particularly puzzling given the similarity in phenotypic response. Body composition changes were similar between twins and most metabolic outcomes were similarly decreased, as one would anticipate with substantial body weight loss. Interestingly, despite very similar reductions in weight and body fat, it was the twin with the higher birth weight who still met the criteria for MetS following the 16-week intervention, due to minimal change in fasting triglycerides from baseline. Hyperlipidaemia can lead to activation of circulating leukocytes via increased fatty acid uptake, and mouse models have shown that MetS increases circulating immune cell counts (Alipour et al., 2008; Kanneganti & Dixit, 2012) and may explain the persistent upregulation of immune-related genes in Twin B compared to Twin A. Excessive fatty acid uptake in monocytes leads to the establishment of foam cells, an initial step in the formation of atherosclerotic plaques (Angelovich et al., 2015; Swirski et al., 2007). We were interested to see whether CRP was reflective of the discordance in fasting triglycerides; however, this was not observed in this case study. CRP is a nonspecific marker of both acute and chronic inflammation, susceptible to multiple environmental factors that influence the immune system (Sproston & Ashworth, 2018). It is therefore probable that other factors, not measured in the current study as such acute illness history, could have impacted measured CRP.

The divergent gene expression changes following weight loss between these twins may be a precursor to more traditional markers of pro-inflammation and may underlie the mechanism behind the MetS only resolving in one twin. Central obesity and the immune system are closely linked (Hotamisligil, 2006). Many metabolic derangements arising from obesity, such as insulin resistance and atherosclerosis, involve inappropriate activation of the immune system. Evidence from both mouse and human studies suggests that an upregulation of immune signalling genes and stress markers on the surface of adipocytes precedes macrophage recruitment to the adipose tissue microenvironment (Bai & Sun, 2015), and the adhesion of monocytes to the endothelium in the initial stages of atherosclerosis development (Wu et al., 2020). Increased expression of the toll-like receptor and the cluster of differentiation (CD) families of genes precedes fibrosis development in mouse adipose tissue following a high fat diet (Kwon et al., 2012). Additionally, individuals with hyperlipidemia have a higher number of primed PBMCs in circulation compared to healthy controls, which precedes adhesion to the endothelium during atherosclerotic plaque formation (Mazor et al., 2008). This suggests that changes in the expression of immune signalling genes may precede metabolic dysfunction and the presence of classical pro-inflammatory markers.

The epigenome is a key regulator of gene expression, inhibiting access of transcriptional machinery to DNA. It has been proposed that differences in the epigenome arise due to epigenetic drift over the life course, and as such the culmination of environmental impacts on gene expression and regulation can result in different phenotypic outcomes (Poulsen et al., 2007). One of the first studies to record this evaluated the epigenomes of 3-year-old and 50-year-old twins and found that epigenetic differences between twin pairs were greater in the older twins (Fraga et al., 2005). These differences were stable over the short term (12 weeks), suggesting long-term exposure is necessary to promote changes in the epigenome (Fraga et al., 2005). Obesity may also promote epigenetic divergence (Barrès & Zierath, 2016) and has been suggested to accelerate biological aging measured through DNA methylation signatures (de Toro-Martín et al., 2019; Nevalainen et al., 2017) and telomere length (Buxton et al., 2011). Differences in the epigenome between twins may lead to differences in gene expression and ultimately phenotypic outcomes, suggesting that the divergent PBMC transcriptomic response observed in the current study may be through DNA methylation differences accumulated through different environmental exposures, which would be an interesting avenue for future exploration.

Monozygotic twins provide a unique model to examine the variability in gene expression following weight loss. This case provides some evidence that changes in the transcriptome may precede the development of more traditional biomarkers of chronic inflammation in susceptible individuals. Further exploration of the transcriptome with weight trajectory over the life course is needed to determine whether expression of genes relating to immune function or alterations in the transcriptome can precede traditional metabolic disease risk factors and to determine what age or developmental stage would be optimal to intervene.

Data availability. Data is available upon reasonable request to the corresponding author.

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Conflict of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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