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Brief Report—Endocrine Research

Blood Cells as a Source of Transcriptional Biomarkers of Childhood Obesity and Its Related Metabolic Alterations: Results of the IDEFICS Study

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Background: IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants Project) is a European multicenter study on childhood obesity. One of its goals is to define early biomarkers of risk associated with obesity and its comorbid conditions.

Objective: We considered blood cells as a new potential source of transcriptional biomarkers for these metabolic disorders and examined whether blood cell mRNA levels of some selected genes (*LEPR*, *INSR*, *CPT1A*, *SLC27A2*, *UCP2*, *FASN*, and *PPARα*) were altered in overweight children and whether their expression levels could be defined as markers of the insulin-resistant or dyslipidemic state associated with overweight.

Design: Blood samples were obtained from 306 normal-weight and overweight children, aged 2–9 yr, from eight different European countries. Whole-blood mRNA levels were assessed by quantitative RT-PCR.

Results: *LEPR*, *INSR*, and *CPT1A* mRNA levels were higher in overweight compared with normal-weight children (the two latter only in males), whereas *SLC27A2* mRNA levels were lower in overweight children. Significant associations were also found between expression levels of *LEPR*, *INSR*, *CPT1A*, *SLC27A2*, *FASN*, *PPARα*, and different parameters, including body mass index, homeostasis model assessment index, and plasma triglycerides and cholesterol levels. These associations showed that high expression levels of *CPT1A*, *SLC27A2*, *INSR*, *FASN*, or *PPARα* may be indicative of a lower risk for the insulin-resistant or dyslipidemic state associated with obesity, whereas low *LEPR* mRNA levels appear as a marker of high low-density lipoprotein cholesterol, independently of body mass index.

Conclusions: These findings point toward the possibility of using the expression levels of these genes in blood cells as markers of metabolic status and can potentially provide an early warning of a future disorder. (***J Clin Endocrinol Metab* 97: E648–E652, 2012**)

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Abbreviations: BMI, Body mass index; Ct, threshold cycle; HOMA, homeostasis model assessment; LDL, low-density lipoprotein.

Obesity in childhood has increased dramatically in the last decades; so have the risks of associated medical conditions, such as type 2 diabetes and cardiovascular disease (1, 2). Because obesity is a multifactorial syndrome influenced by genetic, epigenetic, environmental, and behavioral factors (3, 4) and is often resistant to therapy, early prevention efforts are requested. Therefore, the identification of new early biomarkers of susceptibility is relevant to intervene with lifestyle and nutritional changes before the disease is manifested. In this sense, one of the objectives of the IDEFICS (Identification and Prevention of Dietary- and Lifestyle-Induced Health Effects in Children and Infants Project) is to identify biomarkers of susceptibility and their association with the development of overweight/obesity and metabolic syndrome in European children (5, 6).

Previous studies in rats have pointed out the use of blood cells as a robust model to study energy homeostasis maintenance and its relationship with body weight control (7–9), thus providing a potential tissue source to discover novel biomarkers related with energy balance. The use of blood cells for gene expression studies offers an advantage over other human tissues because they are the most readily accessible tissue and are therefore of great interest for clinical studies and experimental research (7, 10).

Thus, the objective of this study was to evaluate, in blood samples of a subsample of normal-weight and overweight children from the IDEFICS population, whether the expression levels of selected genes involved in energy homeostasis (*LEPR*, *INSR*, *CPT1A*, *SLC27A2*, *UCP2*, *FASN*, and *PPARα*) are altered in the obese state, to identify potential markers of overweight development in children. Additionally, we sought to evaluate whether expression levels of these genes are related with the insulin-resistant or dyslipidemic state associated with overweight.

Subjects and Methods

Subjects

IDEFICS is a large European multicenter study on childhood obesity. Details of the general design and baseline survey characteristics can be found elsewhere (11).

Subjects involved in this study were a subset of 306 children, selected from those enrolled in the baseline survey (that included more than 16,000 children). The subjects included were both males and females, with normal weight and overweight, and belonging to eight European countries geographically spread across Europe: Germany (n = 40), Hungary (n = 29), Italy (n = 43), Cyprus (n = 41), Spain (n = 37), Estonia (n = 37), Sweden (n = 45), and Belgium (n = 34). In all countries, approval from the local ethical committees and parental consent was obtained.

Anthropometric measurements and biochemical measurements

Children in the IDEFICS baseline survey participated in a standardized physical examination. Collected anthropometric data included body weight and height, waist and hip circumference, and skin fold thickness measurement (at four body sites: subscapular, biceps, triceps, and suprailiac). The body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared according to International Obesity Task Force criteria (12). Details on the biological samples collected during the IDEFICS survey have been described elsewhere in detail (13).

Blood sampling and processing for gene expression analysis

For each participant, a total of 2.5 ml peripheral blood was collected under fasting conditions into PAXgene vacutainer tubes (QIAGEN, Hilden, Germany) via antecubital fossa venipuncture, following the manufacturer's instructions (QIAGEN). Total RNA was isolated using the PAXgene blood RNA kit according to the manufacturer's instructions (QIAGEN). RNA quality and purity were analyzed by spectrophotometry using the Nanodrop ND-1000, and RNA integrity was confirmed using agarose gel electrophoresis.

Real-time quantitative RT-PCR analysis

All primers were obtained from Sigma Genosys (Sigma-Aldrich Quimica SA, Madrid, Spain). Quantitative RT-PCR was performed as previously described (14).

The threshold cycle (Ct) was calculated using the instrument's software (StepOne Software version 2.0), and the relative expression ratio of a target gene was calculated based on the corresponding real-time PCR efficiency and Ct deviation of an unknown sample *vs.* mean Ct of all samples and expressed in comparison to a reference gene (15). *Rplpo* was chosen as reference gene because it has been validated for human studies (16).

Statistical analysis

ANOVA was used to determine differences between normal-weight and overweight children and other factors (such as sex, country, and gene expression category) with age as covariate. No interaction between country and the other factors studied was found.

Single differences between two groups were assessed by Student's *t* test. The association between BMI and gene expression in total blood cells was assessed by age-adjusted partial correlation analysis (first order). The median value of the distribution in the overall group was selected as an arbitrary cut point to define gene expression categories (subjects with a low or high expression of *LEPR*, *INSR*, *CPT1A*, *SLC27A2*, *FASN*, and *PPARα*).

All the analyses were performed using SPSS version 18 for Windows (SPSS, Chicago, IL). Threshold of significance was defined at $P < 0.05$.

Results

Characteristics of study subjects

A summary of the subject information is shown in Table 1. Of the 306 children enrolled in the study, 274

TABLE 1. General characteristics, anthropometry, biochemical parameters, and gene expression levels in total blood cells in the population analyzed in the study

	Normal-weight (n = 136)		Overweight (n = 138)		
	Males (n = 68)	Females (n = 68)	Males (n = 65)	Females (n = 73)	ANOVA
Age (yr)	6.6 ± 0.2	6.6 ± 0.2	7.1 ± 0.2	7.1 ± 0.2	W ($P = 0.004$)
Weight (kg)	22.3 ± 0.6	21.5 ± 0.5	38.3 ± 1.0	35.1 ± 0.9	W ($P < 0.001$), S ($P = 0.001$)
Height (cm)	120 ± 1	119 ± 1	130 ± 1	127 ± 1	W ($P < 0.001$), S ($P = 0.001$)
BMI (kg/m^2)	15.3 ± 0.2	15.0 ± 0.2	22.4 ± 0.4	21.6 ± 0.3	W ($P < 0.001$)
sc fat (mm)[a]	22.4 ± 0.7	26.5 ± 1.1	67.0 ± 3.4	66.6 ± 2.9	W ($P < 0.001$)
Waist (cm)	52.7 ± 0.5	51.5 ± 0.5	70.9 ± 1.0	67.9 ± 0.9	W ($P < 0.001$), S ($P = 0.004$)
Waist/hip ratio	0.87 ± 0.01	0.85 ± 0.01	0.90 ± 0.01	0.89 ± 0.01	W ($P < 0.001$), S ($P = 0.029$)
Systolic blood pressure (mm Hg)	103 ± 1	98 ± 1	108 ± 1[b]	107 ± 1[b]	W*S ($P = 0.040$)
Diastolic blood pressure (mm Hg)	64.1 ± 1.0	62.3 ± 0.8	67.0 ± 0.9	66.6 ± 0.8	W ($P < 0.001$)
Glucose (mg/dl)	84 ± 1	82 ± 1	87 ± 1	87 ± 1	W ($P < 0.001$)
Insulin (μIU/ml)	4.31 ± 0.40	4.18 ± 0.33	8.22 ± 0.56	9.53 ± 0.78	W ($P < 0.001$)
HOMA index	0.92 ± 0.09	0.84 ± 0.07	1.83 ± 0.14	2.06 ± 0.18	W ($P < 0.001$)
Triglycerides (mg/dl)	47 ± 3	46 ± 3	52 ± 5	64 ± 5	W ($P = 0.003$)
HDL cholesterol (mg/dl)	57 ± 2	53 ± 2	50 ± 2	47 ± 1	W ($P < 0.001$), S ($P = 0.049$)
LDL cholesterol (mg/dl)	92 ± 5	95 ± 6	104 ± 4	105 ± 3	W ($P = 0.013$)
Total cholesterol (mg/dl)	155 ± 4	159 ± 4	157 ± 4	164 ± 4	
Total/HDL cholesterol ratio	2.97 ± 0.15	3.16 ± 0.11	3.26 ± 0.14	3.67 ± 0.13	W ($P = 0.003$), S ($P = 0.024$)
mRNA levels (%)					
LEPR	88.3 ± 7.2	85.4 ± 6.0	107.6 ± 7.2	101.8 ± 6.9	W ($P = 0.014$)
INSR	86.8 ± 5.6	96.7 ± 6.8	108.7 ± 7.2[b]	96.2 ± 6.7	
CPT1A	93.7 ± 5.4	90.9 ± 6.1	116.8 ± 8.5[b]	84.6 ± 5.8	W*S ($P = 0.027$)
SLC27A2	108.6 ± 8.2	93.7 ± 8.1	73.7 ± 5.6	81.1 ± 7.7	W ($P = 0.003$)
PPARα	99.2 ± 5.9	96.6 ± 6.1	104.0 ± 6.0	97.0 ± 5.5	
FASN	92.9 ± 4.6	96.4 ± 5.7	107.5 ± 6.6	93.7 ± 5.5	
UCP2	93.5 ± 5.2	100.3 ± 7.2	101.2 ± 6.8	95.5 ± 6.2	

Data are presented as mean ± SEM. Statistical analysis, W*S represents the interactive effect between BMI categories and sex ($P < 0.05$, two-way ANOVA); S represents the differences between males and females ($P < 0.05$, two-way ANOVA); W represents differences between overweight and normal weight ($P < 0.05$, two-way ANOVA). All ANOVA were corrected for age. HDL, High-density lipoprotein.

[a] Subcutaneous fat represents the sum of four different skin folds (subscapular, biceps, triceps, and suprailiac).

[b] Differences between overweight and normal weight within the same sex ($P < 0.05$, Student's *t* test).

subjects were fully characterized. Weight categories were defined according to Cole *et al.* (12). Of note, despite being very young, overweight children already showed significant differences compared with normal-weight children in parameters tested [higher sc fat, waist, waist to hip ratio, systolic and diastolic blood pressure, and homeostasis model assessment (HOMA) values] as well as exhibited higher circulating concentrations of insulin, glucose, triglycerides, low-density lipoprotein (LDL) cholesterol, and lower high-density lipoprotein cholesterol.

Gene expression levels in total blood cells

Transcript levels of *LepR* were significantly higher in blood cells from overweight compared with normal-weight children (Table 1). Concerning *INSR* and *CPT1A* transcripts, there was an interaction between gender and BMI category ($P = 0.092$ and 0.027, respectively, by ANOVA); male overweight children presented higher expression levels, whereas no differences were found in female children. The transcript levels of *SLC27A2* were lower in both male and female overweight children compared with normal-weight children. Transcript levels of *PPARα*, *FASN*, and *UCP2* were not significantly different between overweight and normal-weight children.

Association studies of gene expression with anthropometric and biochemical parameters of subjects

To examine the potential interaction between gene expression in blood cells and BMI, LDL cholesterol, triglycerides, and HOMA index, subjects were subdivided into categories according to gene expression levels (low *vs.* high). When subjects were subdivided on the basis of *LEPR* expression levels, normal-weight children with low expression levels of *LEPR* exhibited higher levels of LDL-cholesterol than normal-weight children with high levels of *LEPR* transcripts, and similar to those found in overweight children (Fig. 1A). No significant differences were found concerning LDL cholesterol levels in overweight children in relation to their levels of *LEPR* transcripts.

Concerning triglyceride levels, overweight children with high expression levels of *INSR* or *CPT1A* did not show the increase in triglyceride levels associated with fat

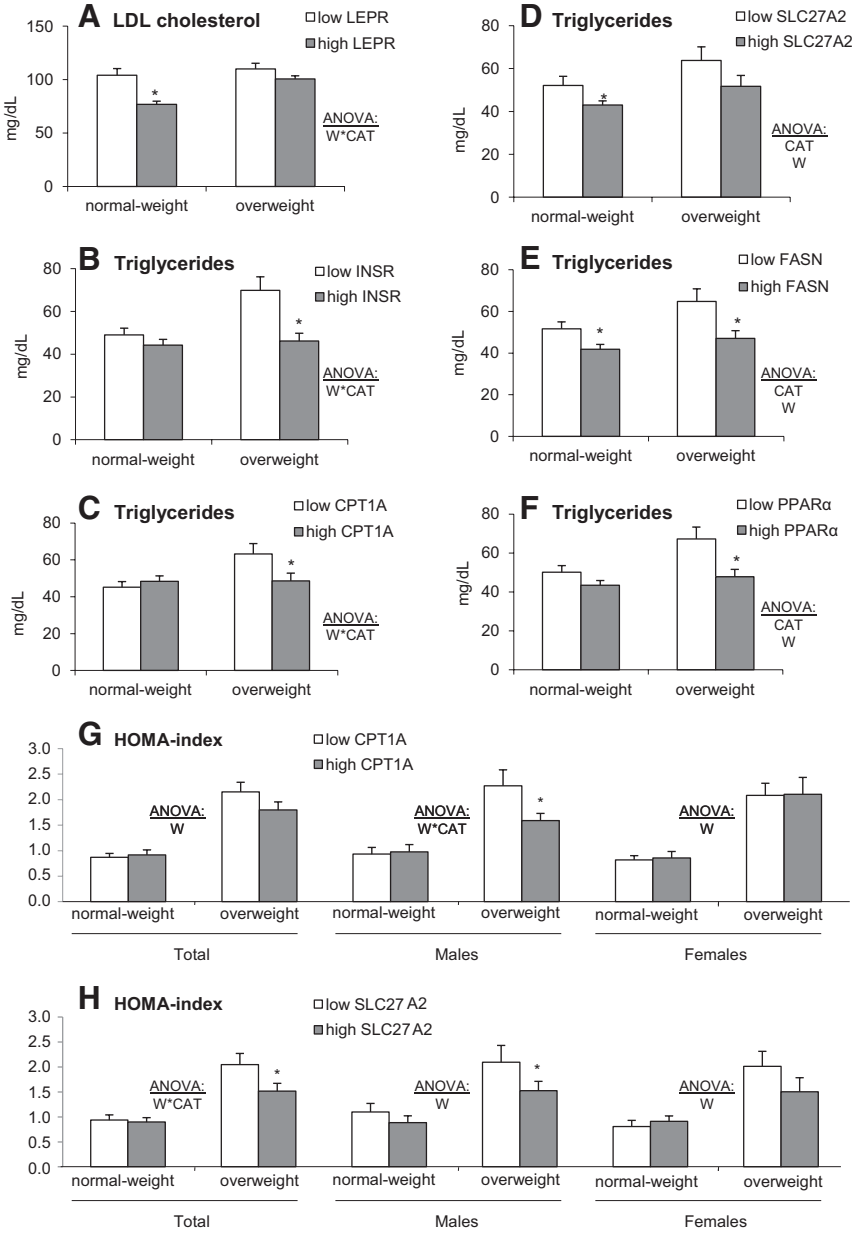

FIG. 1. Comparison of plasma LDL cholesterol (A) and triglyceride levels (B–F) and HOMA index (G, H) in normal-weight and overweight children with low and high mRNA expression levels of different genes in total blood cells. Results are mean ± SEM. W*CAT, Interactive effect between BMI categories and categories of gene expression ($P < 0.05$, two-way ANOVA); CAT, differences between groups divided by gene expression categories ($P < 0.05$, two-way ANOVA); W, differences between overweight and normal-weight ($P < 0.05$, two-way ANOVA); *, differences between low and high gene expression within a same BMI group ($P < 0.05$, Student's *t* test). All ANOVA analyses were corrected for age.

accumulation (Fig. 1, B and C), and both normal-weight and overweight children with high expression levels of *SLC27A2*, *FASN*, or *PPARα* exhibited lower triglyceride levels (Fig. 1, D–F).

Regarding HOMA index, the negative effect of being overweight appears to be attenuated by high *CPT1A* mRNA levels in male individuals and by high *SLC27A2* mRNA levels in both males and females but with a more marked effect in males (Fig. 1, G and H).

Discussion

For early detection and prevention of overweight-associated diseases, it is necessary to develop new early biomarkers. Blood can be easily obtained from human subjects, and therefore gene expression patterns in blood cells can be easily measured for screening purposes. Here, we focus on the study of the expression of genes related to glucose and lipid metabolism in total blood cells, to discover novel biomarkers related to energy balance in children, because we postulate that blood cell expression pattern may reflect the gene expression profile of key tissues involved in energy metabolism, such as the liver or the adipose tissue, as previously pointed out (7).

Transcript levels of leptin and insulin receptor were found to be overexpressed in children with overweight, although in the case of *INSR*, the increase was found only in male children. Concerning *LEPR*, we found a negative correlation (after correction for confounding factors BMI and age) between the expression levels of *LEPR* in blood cells and levels of cholesterol (both LDL and total) (partial $r = -0.275$ and -0.216, respectively, $P < 0.01$). Noteworthy, normal-weight children with low levels of *LEPR* transcripts had similar levels of LDL cholesterol as overweight children. Then, independently of BMI, lower expression levels of *LEPR* in blood cells may reveal high LDL cholesterol levels. With a similar pattern, *INSR* transcript levels were negatively correlated with triglycerides, after correction for confounding factors BMI and age (partial $r = -0.266$, $P < 0.01$), and when individuals were classified by high and low *INSR* expression, we found that those overweight individuals with high expression levels of this gene seem to be protected against the hypertriglyceridemia associated with obesity.

Concerning *CPT1A*, we found that this gene was overexpressed in males with overweight but not in females. Higher expression levels of *CPT1A* in blood cells have also been described in diet-induced obese rats (9), and this gene is also overexpressed in the liver of obese male rats, but not in females (17). Interestingly, and similarly to results ob-

tained with *INSR*, overweight individuals with high expression levels of *CPT1A* in blood cells seem to be protected against the increase in plasma triglyceride levels associated with body fat accumulation. In addition, we found a negative correlation (after correction for BMI and age) between *CPT1A* mRNA levels and HOMA index only in male children (partial $r = -0.284$, $P < 0.05$). Moreover, within overweight males, those with lower expression levels of this gene showed higher HOMA index than those with higher expression levels. Then, *CPT1A* expression levels in blood cells appear to be a good marker for the insulin-resistant state associated with overweight/obesity in male children.

SLC27A2 expression levels were lower in overweight compared with normal-weight children. The decrease of *SLC27A2* expression levels in blood cells in obese children together with the negative correlation coefficients found between the expression of this gene in blood cells and BMI (partial $r = -0.214$, $P < 0.01$) make this gene a candidate biomarker of obesity/overweight in children. In addition, regardless of body weight, individuals with high mRNA levels of *SLC27A2* in blood cells displayed lower triglyceride levels. Moreover, within overweight children, those with lower expression levels of this gene exhibited higher HOMA values than those with higher expression levels. Thus, low expression levels of *SLC27A2* (as observed for *CPT1A*) seem to exacerbate the insulin-resistant state associated with excess fat accumulation, particularly in males.

We did not find differences between normal-weight and overweight children concerning the expression levels of *FASN* and *PPARα* in blood cells, but curiously, children with high mRNA levels of these genes in blood cells displayed lower triglyceride levels, regardless of body weight, similarly to what was observed for *SLC27A2*.

It may be interpreted from the findings of this study that some overweight children may be at an early stage of the pathogenesis of diabetes and dyslipidemia, and the expression levels of specific genes in blood cells could be used as markers of body metabolic status, potentially providing an early warning of future disorders. Further investigation in longitudinal studies will help to determine later consequences of these identified biomarkers.

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