TAS1R3 and *UCN2* transcript levels in blood cells are associated with sugary and fatty food consumption in children

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Context: New types of dietary exposure biomarkers are needed to implement effective strategies for obesity prevention in children. Of special interest are biomarkers of consumption of food rich in simple sugars and fat, as their intake has been associated with obesity development. Peripheral blood cells (PBCs) represent a new promising tool for identifying novel transcript-based biomarkers.

Objective: To study potential associations between the transcripts of taste-receptor-type-1-member-3 (*TAS1R3*) and urocortin II (*UCN2*) genes in PBCs and the frequency of sugary and fatty food consumption in children.

Design, setting and participants: 463 children from the IDEFICS cohort selected to include similar number of boys and girls, with normal-weight and overweight, belonging to eight European countries.

Main outcome measures: Anthropometric parameters (measured at baseline and in a subset of 193 children after two years), food consumption frequency and transcript levels of *TAS1R3* and *UCN2* genes in PBCs.

Results: Children with low frequency consumption of sugary foods displayed higher *TAS1R3* expression levels with respect to those with intermediate or high frequency. In turn, children with high frequency consumption of fatty foods showed lower *UCN2* expression levels with respect to those with low or intermediate frequency. Moreover, transcripts of *TAS1R3* were related with BMI and fat-mass changes after a two-year follow-up period, with low expression levels of this gene being related with increased fat accumulation overtime.

Conclusion: The transcripts of *TAS1R3* and *UCN2* in PBCs may be considered as potential biomarkers of consumption of sugary and fatty food, respectively, to complement data of food-intake questionnaires.

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Abbreviations:

Childhood is a critical life period for the establishment of dietary habits, which may affect nutrition and health status later in life (1). Individual preferences and consumption of palatable high-energy foods, such as those rich in fat or sugars, during early ages have been associated to obesity and its related complications (2–6). In this regard, different scientific advisory bodies gave specific advice for the consumption of fat and sugar (7–9). However, to establish coherent, effective food-based strategies to prevent obesity in children, more precise measures of actual intake of sugary and fatty foods are needed. In this sense, identification of biomarkers related to food consumption patterns may provide more objective measures to complement data of food intake questionnaires.

Quantification of nutrition, meaning not only what is ingested but also its biological effects, is hindered by the lack of appropriate biomarkers. At present, there are few biomarkers of food exposure, and the existing ones - with the exception of some nutrients - are generally very imprecise in measuring real intake (10). Nutrigenomic-based technologies offer new tools to address this deficiency (11). Concretely, the transcriptional profile of peripheral blood cells (PBCs), using whole blood cells or the purified fraction of peripheral mononuclear cells (PBMCs), has been proposed as a useful instrument to assess the physiological and nutritional effects of food (12, 13). Blood cells offer the advantage, over other human tissues, of being easily accessible from blood samples, while reflecting the transcription profile occurring in other tissues (14, 15). Associations between food consumption and the transcriptional profile of PBCs have been previously reported in a microarray study, showing that gene expression profiles in PBMCs from healthy humans were different according to dietary patterns (13). There are also several studies showing changes in transcriptomic profile of PBMCs after consumption of diets rich in n3 PUFAS (16–18) and other dietary modifications (reviewed in (19)). In children, expression levels of specific genes in PBCs (using whole blood cells) have been proposed as biomarkers of the metabolic status, as they are indicative of the risk of the insulin resistant or dyslipidaemic state associated with obesity (20). Gene expression in PBCs of children has also been shown to be related with the type of feeding during lactation, and may be indicative of the protective effects of breastfeeding against obesity and other metabolic alterations (21).

Here we aimed to examine potential associations between transcript levels of candidate genes in PBCs and the frequency of consumption of food rich in simple sugars and fat to identify new biomarkers of exposure to these types of food. Selected candidate genes were *taste receptor type 1 member 3* (*TAS1R3*), for sugary food, and *urocortin II* (*UCN2*), for fatty food. The selection of genes was based on existing literature suggesting their association with sensitivity to and/or preference for sugar or fat (22, 23), and on our preliminary assays showing significant expression of these genes in PBCs. *TAS1R3* gene codifies for a sweet taste receptor (*TAS1R3*), and single-nucleotide polymorphisms in the human *TAS1R3* gene have been associated with differences in sucrose taste sensitivity (22). In turn, *UCN2* encodes for urocortin 2 (*UCN2*), which belongs to a family of corticotrophin releasing factor peptides with an important role in the control of food intake (23). This peptide seems to be related to the preference for high-fat food but with controversial results. Concretely, in rats, expression levels of *UCN2* in hypothalamus have been correlated with the preference for high-fat diet (24), but when centrally administered, it has been shown to produce a significant decrease in the intake of high-fat diet (25). Thus we studied potential associations between transcript levels of *TAS1R3* and *UCN2* in PBCs and the frequency of consumption of sugary and fatty food, respectively, in a sample of children from the IDEFICS cohort (26). In addition, the predictive value of the expression of these genes on the risk of body fat accumulation over time was ascertained in a two-year follow-up of a subgroup of the study population.

Subjects and Methods

Participants

Subjects involved in the study were a sample of 463 children from the IDEFICS cohort and aged between 2 and 11 years. The participants were selected to include a similar number of both boys and girls, with normal weight and overweight, and belonging to the eight European countries involved in the IDEFICS project as survey centers (Germany, Hungary, Italy, Cyprus, Spain, Estonia, Sweden and Belgium). A subset of children (193; 89 boys and 104 girls) was also examined after a follow-up period of 2 years.

Approval by the appropriate ethics committees was obtained by each of the eight participating centers carrying out the fieldwork. Participants were not subjected to any study procedure before both the children and their parents gave their oral (children) and written (parents) informed consent for examinations, collection of samples, subsequent analysis and storage of personal data and collected samples. Details concerning the biological samples collected and processed for the IDEFICS survey have been described elsewhere in detail (27).

Anthropometric measurements

Children in the IDEFICS surveys underwent a standardized physical examination. Anthropometric data included body weight and height, waist circumference and the measurement of skinfold thickness. A detailed description of the anthropometric measurements adopted in the IDEFICS study, including intra- and interobserver reliability, has been published (28). The mea-

surement of weight was carried out using an electronic scale (Tanita BC 420 SMA, Tanita Europe GmbH, Sindelfingen, Germany) to the nearest 0.1 kg with children wearing light clothes and without shoes. Height was measured using a telescopic height measuring instrument (Seca 225 stadiometer, Birmingham, UK) to the nearest 0.1 cm. BMI was calculated as weight (in kg) divided by height squared (in m). Waist circumference was measured using an inelastic tape (Seca 200, Birmingham, UK), precision 0.1 cm, at the midpoint between the iliac crest and the lower coastal border or 10th rib with the subject in a standing position and recorded at the nearest 0.1 cm. Both triceps and subscapular skinfold thickness ware measured by means of a caliper (Holtain, Holtain Ltd,Pembrokeshire, UK, range 0640 mm). Measures were taken twice on the right hand side of the body and the mean was calculated. For the definition of overweight/obesity, children were grouped into two categories using the cut-points defined by Cole et al (29).

Dietary assessment

Food consumption frequency was estimated by the Children' s Eating Habits Questionnaire (CEHQ -FFQ) (30), in which parents or another proxy living with the child report the frequency of their child' s consumption of selected food items in a typical week during the preceding 4 weeks, outside the school canteen or childcare meal provision settings. CEHQ -FFQ asked for the consumption frequency of 43 pan-European food items of 14 food groups. Response options were as follows: 'Never/less than once a week', '1–3 times a week', '4–6 times a week', '1 time per day', '2 times per day', '3 times per day', '4 or more times per day' and 'I have no idea'. Frequency categories were converted into times per week ranging from 0 to 30. The response "I have no idea" was treated as missing. Children with more than 50% missing food items were excluded of the calculation. Consumption frequencies of simple sugar (or sugary) and fatty food were considered in the analysis. The types of foods included in both categories and the weekly frequency of consumption corresponding to the 25th and 75th percentiles are indicated in Table 1.

For association studies, dietary assessment data of sugary and fatty food frequencies were classified into three categories according to percentiles: low consumption (below the 25th percentile), intermediate consumption (between 25–75th percentiles) and high consumption (above the 75th percentile).

A separate 24-hour recall module was used to estimate energy and macronutrient intake. This was performed using the computer-assisted 24-hour dietary recalls (24-HDR), called SACINA ('Self Administered Children and Infant Nutrition Assessment') (31). Parents or other caregivers as proxy respondents for recalling children's diet required information on amount (g) and type of all foods and drinks that were consumed during the previous day, starting with the first intake after waking up in the morning. Accurate estimation of portion size was assisted using standardized photographs. School meals, drinks and snacks consumed the day prior to the 24-HDR were assessed using a standardized observer sheet, completed by trained personnel.

Real-time quantitative RT-polymerase chain reaction (PCR) (RT-qPCR) analysis in whole blood cells

Reverse transcription quantitative PCR (RT-qPCR) was used to measure mRNA expression levels as previously described (20). In short, a total of 2.5 mL of peripheral blood was collected under fasting conditions into PAXgene vacutainer tubes via antecubital fossa venipuncture, following the manufacturer's instructions (Qiagen, Hilden, Germany). Total RNA was isolated using the PAXgene blood RNA kit according to the manufacturer's instructions (Qiagen). Primers were obtained from Sigma Genosys (Sigma-Aldrich Quimica SA, Madrid, Spain). The threshold cycle (Ct) was calculated by the instrument's software (StepOne Software v2.2.2) and the relative expression of each mRNA was calculated using the $2^{-\Delta\Delta Ct}$ method. *Trim27* was used as a housekeeping gene for PCR normalization (32). The suitability of this gene as a housekeeping was also confirmed.

Statistical analysis

Statistical analysis was performed using SPSS (version 20). Data are presented as mean and the standard deviation. One-way ANCOVA was used to assess differences between groups divided according to the percentile of distribution, adjusted for age and BMI. Bonferroni post hoc test was used when differences were statistically significant. When indicated, ANCOVA analysis was also adjusted for food consumption frequency variables. The Chi-Square test was used to compare proportions for categorical variables, and Student's *t* test was used to compare two means of continuous variables. Threshold of significance was defined at $P < .05$.

Results

Characteristics of the population

463 children from the IDEFICS cohort were studied. Characteristics of the study population are presented in Table 2. The sample studied included boys and girls, with

Table 1. Frequencies of consumption of sugary and fatty food, expressed in times per week, corresponding to the 25th and 75th percentiles in the study population. The types of foods included in each category is indicated.

Food category	Frequency consumption		Types of food
	25th	75th	
Sugary food	15	33	Fruit juices, sweetened drinks, sugar added cereals, sweetened milk, sweetened yoghurt, and four types of snacks, like chocolate bars, candies, cakes, or ice cream
Fatty food	2	7	Fried potatoes, fried fish, fried meat, fried or scrambled eggs

Table 2. Characteristics of the study population. Data are presented as mean and standard deviation (in brackets). AU, arbitrary units; BMI, body mass index; NW, lean/normal weight; OW, overweight/obese; TAS1R3, taste receptor type 1 member 3; UCN2, urocortin II. Statistics: *, differences between NW and OW ($P < 0.05$ by Student's *t* test).

	All participants	Boys	Girls	NW	OW
	$n = 463$	$n = 224$	$n = 239$	$n = 222$	$n = 241$
Anthropometric parameters					
BMI (kg m-2)	18.9 (4.2)	19.1 (4.4)	18.8 (4.0)	15.4 (1.5)	22.2 (3.1) *
Body fat (%)	34.3 (9.3)	31.9 (9.6)	36.5 (8.4)	26.8 (6.3)	41.3 (5.4) *
Waist circumference (cm)	62.3 (11.1)	62.9 (11.6)	61.8 (10.7)	53.5 (4.8)	70.4 (8.9) *
Sum of skinfolds (mm)	25.5 (12.9)	24.1 (12.9)	26.8 (12.7)	15.1 (3.9)	35.2 (10.5) *
Energy intake					
kcal/day	1577 (562)	1629 (583)	1527 (538)	1602 (569)	1549 (555)
% of energy from carbohydrate	53.0 (11.5)	52.7 (11.7)	53.3 (11.3)	52.5 (11.1)	53.5 (12.0)
% of energy from fat	32.6 (9.0)	32.9 (9.1)	32.3 (8.9)	33.2 (9.3)	31.9 (8.7)
% of energy from protein	15.5 (4.5)	15.3 (4.0)	15.6 (4.9)	15.3 (4.3)	15.7 (4.7)
Food intake frequency (times per week)					
Sugary food	25.4 (14.5)	26.3 (15.8)	24.5 (13.2)	26.1 (14.2)	24.7 (14.9)
Fatty food	5.2 (3.8)	5.1 (3.8)	5.3 (3.9)	5.4 (4.0)	5.0 (3.7)
mRNA levels (AU)					
TAS1R3	81.8 (142)	84.2 (149)	81.1 (135)	80.3 (134)	84.7 (150)
UCN2	27.8 (45.1)	31.6 (44.5)	27.7 (45.6)	32.0 (46.8)	27.5 (43.4)

lean/normal weight (NW) and overweight/obese (OW), in similar proportions. The mean age of all participants was 7.5 years (range 2.3 to 11.6).

Mean BMI, percentage of body fat, waist circumference and sum of subscapular and triceps skinfolds did not differ between boys and girls participants. These variables were greater in the OW children in comparison with the NW ones (Table 2). The mean total energy intake (1577 kcal/d) and the percent of energy from each macronutrient category, as well as the mean frequency of sugary and fatty food consumption did not differ between boys and girls or between NW and OW children (Table 2).

Expression levels of *TAS1R3* and *UCN2* were not different between boys and girls. No significant differences in transcript levels of these genes were found between NW and OW children either (Table 2).

Association studies between gene expression in PBCs and food consumption frequency

Figure 1 shows the expression levels of *TAS1R3* and *UCN2* in PBCs in the study population classified according to percentiles of consumption frequencies of sugary and fatty food (low, intermediate and high, representing < 25th, 25–75th and > 75th percentiles, respectively). Expression levels of *TAS1R3* were associated with the frequency of consumption of food rich in simple sugars. As shown in Figure 1A, children with low frequency consumption of food rich in simple sugars showed higher expression levels of *TAS1R3* with respect to those with intermediate or high frequency.

Expression levels of *UCN2* were associated with the frequency of consumption of fatty food (Figure 1B). Concretely, children with high frequency consumption of fatty food showed lower expression levels of *UCN2* with respect to those with low or intermediate frequency consumption.

Thus, expression levels of *TAS1R3* and *UCN2* in PBCs appear to be related to the frequency of consumption of sugary and fatty food. To estimate their potential usefulness as biomarkers of consumption of these types of food, the same population of children was divided into three categories according to gene expression levels (low, intermediate and high, representing < 25th, 25–75th and > 75th percentiles, respectively) and the frequency of consumption of these specific types of foods (Table 3). Significant associations were found between *TAS1R3* expression levels and the frequency of consumption of sugary food ($P < .01$, Chi-Squared test), and between *UCN2* expression levels and frequency consumption of fatty food ($P < .01$, Chi-Squared test). Concerning *TAS1R3*, most of the children with low expression levels of this gene showed intermediate or high frequency consumption of sugary; only 16% of them showed low frequency consumption of sugary food. Conversely, children with high expression levels showed low or intermediate frequency consumption of these types of food; only 20% of them showed high frequency consumption of sugary food. Intermediate levels of expression of *TAS1R3* did not seem to allow a clear discrimination of the frequency of consumption of this type of food. A similar association to that of *TAS1R3* with sugary food was found between *UCN2* expression levels and fatty food.

Association studies between gene expression in PBCs and anthropometric parameters

Considering the above-described association between expression levels of *TAS1R3* and *UCN2* in PBCs and the frequencies of consumption of sugary and fatty food, we next explored the potential relationship between expression levels of both genes and adiposity-related parameters in the basal study population. As shown in Table 4, no significant differences were found concerning BMI, percentage of body fat, waist circumference or sum of triceps and subscapular skinfolds between the three categories of children classified according to percentiles of expression of *TAS1R3* and of *UCN2*. However, as expected, frequencies of sugary food consumption were different between groups classified according to *TAS1R3* mRNA expression, and frequencies of fatty food consumption were different between groups classified according to *UCN2* mRNA expression (Table 4).

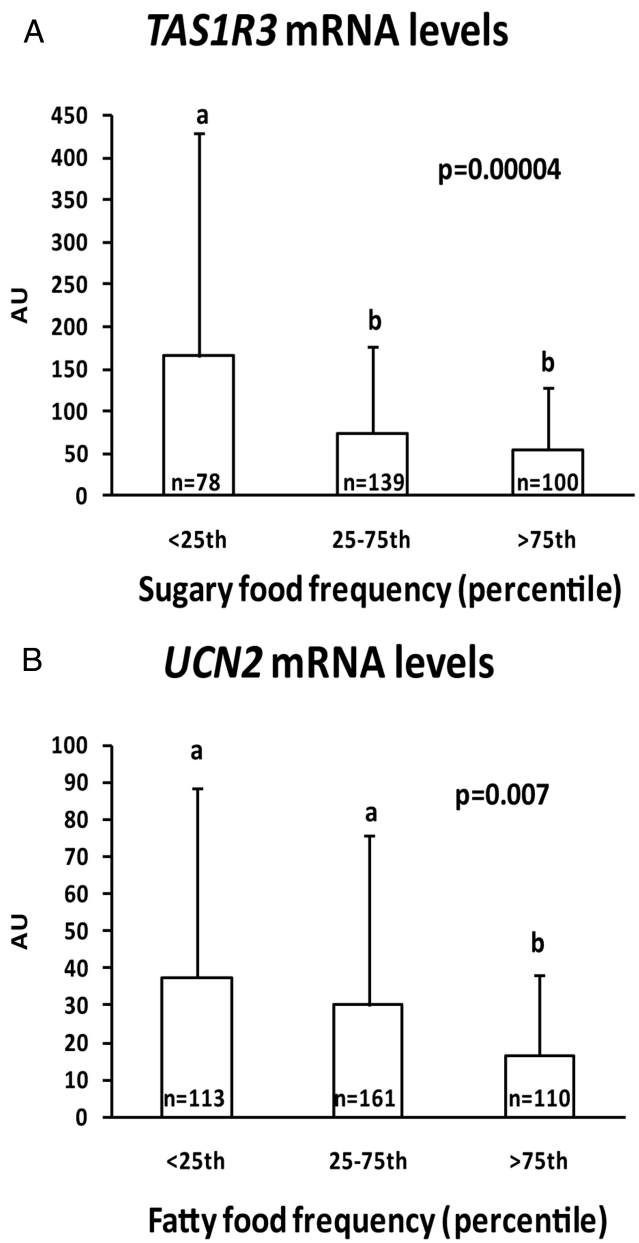

Figure 1. Expression levels of TAS1R3 (A) and UCN2 (B) in peripheral blood cells in the study population spread according to consumption frequencies (<25th, 25–75th and > 75th percentiles) of sugary food (for TAS1R3, taste receptor type 1 member 3) and fatty food (for UCN2, urocortin II). Results are mean ± standard deviation expressed in arbitrary units (AU). The number of children in each group is indicated. Statistics: a≠b by Bonferroni post hoc analysis; p-values of one-way ANCOVA are indicated.

Association studies between gene expression in PBCs and anthropometric parameters in a two-year follow-up

To explore the predictive value of the expression levels of *TAS1R3* and *UCN2* in PBCs on the risk of obesity development, the changes in anthropometric parameters (BMI, percentage of body fat, waist circumference, and sum of triceps and subscapular skinfolds) over the two-year follow-up were used. These data were available for a subgroup of the study population (193 children). The expression levels of *TAS1R3* in PBCs of children (divided into three categories according to percentiles) were significantly associated with the two-year variation of anthropometric parameters (**Figure 2**). Children with low expression levels of *TAS1R3* (<25th percentile) showed a greater increase in BMI (Figure 2A), waist circumference (Figure 2C) and sum of skinfolds (Figure 2D) compared to children with expression levels above the 25th percentile. These associations were lost when the analyses were performed adjusting by food consumption variables. No significant associations were found between the expression levels of *UCN2* in PBCs and the above mentioned parameters (data not shown).

Notably, no significant associations were found between the frequencies of consumption of sugary or fatty food and the two-year variation in the anthropometric parameters above mentioned (data not shown).

Discussion

The development of new dietary-related biomarkers is crucial for nutrition research to enable a more accurate and objective assessment of food intake and to establish effective food-based strategies to prevent obesity. Analysis of PBCs transcriptomic profile is emerging as a useful tool for this purpose (13). Most of blood cells gene expression studies have been performed in a specific subpopulation, the peripheral blood mononuclear cells (PBMCs), which include lymphocytes and monocytes and constitutes a reliable and homogeneous sample for transcriptome analysis (13, 16, 19). However, the technical procedures for the

Table 3. Percentage and number (in brackets) of children with low, intermediate and high (<25th, 25–75th and >75th percentiles, respectively) expression levels of TAS1R3 (taste receptor type 1 member 3) and of UCN2 (urocortin II) in peripheral blood cells and spread according to frequencies of consumption of sugary and fatty food. Statistics: p-values of χ-squared test ($\chi 2$) are indicated.

	Sugary food frequency			$\chi 2$
Expression levels of *TAS1R3*	**low (<25th)**	**intermediate (25–75th)**	**high (>75th)**	**p**
low (<25th)	16.3% (13)	43.8% (35)	40.0% (32)	
intermediate (25–75th)	20.6% (32)	45.8% (71)	23.5% (52)	0.001
high (>75th)	40.2% (33)	40.2% (33)	19.5% (16)	
	Fatty food frequency			$\chi 2$
	low (<25th)	intermediate (25–75th)	high (>75th)	p
Expression levels of *UCN2*				
low (<25th)	18.3% (17)	39.8% (37)	41.9% (39)	
intermediate (25–75th)	27.6% (54)	43.4% (85)	29.1% (57)	0.00 009
high (>75th)	44.2% (42)	44.1% (39)	14.7% (14)	

Table 4. Anthropometric parameters and food intake frequencies of consumption of sugary and fatty food of the study population spread according to expression levels (<25th, 25–75th and >75th percentiles) of TAS1R3 (taste receptor type 1 member 3) and of UCN2 (urocortin II). Results are mean and standard deviation (in brackets). Statistics: a≠b by Bonferroni post-hoc analysis.

	TAS1R3 mRNA levels			UCN2 mRNA levels		
	low (<25th)	**intermediate (25–75th)**	**high (>75th)**	**low (<25th)**	**intermediate (25–75th)**	**high (>75th)**
			Anthropometric parameters			
BMI (kg m-2)	**19.3 (4.2)**	**18.9 (4.2)**	**18.3 (4.3)**	**19.3 (4.2)**	**18.6 (4.2)**	**19.1 (4.2)**
Body fat (%)	35.0 (8.8)	34.5 (9.5)	32.9 (9.2)	35.5 (9.8)	33.6 (9.2)	34.6 (8,.9)
Waist circumference (cm)	63.6 (11.1)	62.3 (11.1)	60.4 (11.2)	63.0 (11.3)	61.7 (11.2)	62.4 (10.8)
Sum of skinfolds (mm)	27.2 (13.3)	25.5 (13.1)	23.2 (11.6)	27.5 (13.1)	24.4 (12.6)	25.5 (12.9)
Food intake frequency (times per week)						
Sugary food	28.6 (15.3) a	25.7 (12.7) a	19.7 (12.8) b	24.9 (12.8)	25.6 (15.1)	24.2 (14.8)
Fatty food	5.7 (3.9)	5.4 (3.9)	4.6 (3.6)	6.1 (3.8) a	5.3 (4.0) a	4.0 (3.2) b

isolation of PBMCs require several methodological steps that must be strictly followed and performed immediately after blood collection to avoid ex vivo changes in gene expression profile. This may cause several logistic and technical problems, particularly when involve multicenter studies. Alternative technical procedures, such as the PAXgene blood RNA system, used in this study, allow the collection and stabilization of RNA from whole blood cells immediately upon blood sampling without the need of further manipulations (33). Thus, this procedure offers a number of technical advantages, such as the easy way of collection, storage and transport of samples, or the reduced time of sample manipulation, that make this procedure easily standardized and highly reproducible, and hence represent an attractive approach for multicenter studies (34). The limitation of procedures using whole blood cells is that they do not permit the sorting of specific cell populations since all types of blood cells are lysed in the process. In addition, some studies have shown increased noise and reduced responsiveness with whole blood cells in comparison to PBMCs (35). Nevertheless a significant overlap between whole blood (using PAXgene tubes) and PBMC gene expression has been demonstrated (36, 37), and hence it is expected that biomarkers identified using whole blood cells may be extended to PBMCs.

The results of the present study, using whole blood

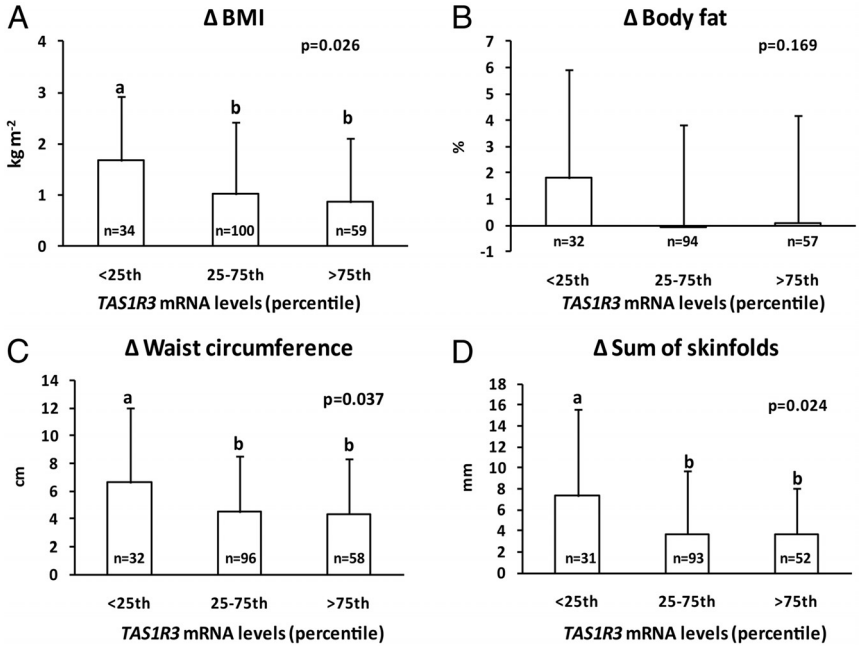

Figure 2. Changes (Δ) in anthropometric parameters (A: body mass index (BMI); B: percentage of body fat; C: waist circumference; and D: sum of skinfolds) over 2 years in a subset of the study population spread according to expression levels of taste receptor type 1 member 3 (TAS1R3) (<25th, 25–75th and > 75th percentiles). Results are mean ± standard deviation. The number of children in each group is indicated. Statistics: a≠b by Bonferroni post hoc analysis; p-values of one-way ANCOVA are indicated.

cells, show that the expression levels of *TAS1R3* and *UCN2* in PBCs of children are associated with frequencies of consumption of groups of foods potentially related to obesity development. Concretely, expression levels of *TAS1R3* are associated with frequency of sugary food consumption, while expression levels of *UCN2* are associated with frequencies of fatty food consumption.

TAS1R3 gene codifies for a sweet taste receptor (*TAS1R3*, taste receptor type 1 member 3) that forms a heterodimer with TAS1R2 to recognize sweet-tasting molecules (38). Low expression levels of *TAS1R3* in taste bud cells have been related to lower sensitivity to the sweet flavor (39), but notably, knock-out mice for this receptor have a high preference for sucrose (40, 41). Thus, it seems that low sensitivity for sweet flavor may favor higher consumption of sweet-tasting food. Our findings showing that children with low frequency consumption of sugary food display higher expression levels of *TAS1R3* in PBCs in comparison to children with intermediate or high frequencies, suggest that expression levels of this gene in PBCs may be indicative of the frequency of sugary food consumption. From the present results it can be deduced that it is unlikely that children with high expression levels of this gene in PBCs (> 75th percentile) will show a frequency of consumption of sugary food above the 75th percentile (> 33 per week). Conversely, low expression levels of *TAS1R3* (<25th percentile) may be indicative of intermediate or high frequency of consumption of food rich in simple sugars (> 15 times per week).

UCN2 is one of the most potent agonists of corticotropin-releasing factor (CRF) 2 receptor (CRF2) and is implicated in food intake and anxiety-like behavior (23). It seems that *UCN2* is involved in the preference for high-fat food; high expression levels of *UCN2* in the hypothalamus have been found to be positively correlated with the preference for high-fat food in rats (24). However, when centrally administered, this protein has been shown to decrease high-fat diet intake, not only in lean, but also in diet-induced obese rats (25), as well as to reduce the overeating of palatable cafeteria diet (42). Notably, our results show that children with high frequency consumption of fatty food (above the 75th percentile) display low expression levels of *UCN2* with respect to those with low or intermediate frequencies. Therefore, analogously to the case of *TAS1R3* with sugary food, low expression levels of *UCN2* may be indicative of a frequency of fatty food consumption above the 25th percentile (in this case more than 2 times per week).

Thus, *TAS1R3* and *UCN2* expression levels in PBCs are related with the frequency of consumption of sugary and fatty food, respectively, and hence may be considered as potential biomarkers of the consumption of these types of food. In both cases, their usefulness to correctly classify individuals according to the frequency of consumption of sugary and fatty food, respectively, seems to be of particular value for low or high expression levels of this gene.

Biomarkers able to assess the excessive consumption of sugar- and fat-rich food are of high value to implement early strategies for obesity prevention, because it has been described that overconsumption of both groups of foods may contribute to the obesity epidemic (43, 44). This kind of biomarkers, in combination with questionnaires, may help to avoid confounding factors related to human subjective nature and deserves special interest in the case of children. Interestingly, we show here that expression levels of *TAS1R3* in PBCs may predict changes in body composition in a two-year follow-up. This association was lost when the analysis was adjusted by food-consumption-frequency variables, indicating that this relationship is dependent on the children's food intake. In this sense, chil-

dren with low expression levels of *TAS1R3* in PBCs (below the 25th percentile) underwent the greatest increase in BMI, waist circumference and skinfolds compared to children with higher levels of expression. Unlike *TAS1R3*, expression levels of *UCN2* were not significantly related to changes in the above-mentioned anthropometric parameters. In the current study, it is worth noting that no significant associations were found when data from questionnaires on sugary or fat frequency were used instead of data on gene expression in the follow-up study. This suggests that the measurement of the expression levels of *TAS1R3* in PBCs might reflect more accurately the frequencies of consumption of specific types of undesirable food than the questionnaires, particularly in terms of adverse effects on body weight and fat accumulation. A limitation of the study is the fact of having fewer children in the follow-up study, as well as the lack of information on potential changes in food intake habits during this period. Further studies in other populations would be interesting to confirm these associations.

In summary, the results of the present study show that *TAS1R3* and *UCN2* expression levels in PBCs are related to the frequency of consumption of sugary food (case of *TAS1R3*) and of fatty food (case of *UCN2*) in children, and hence may be considered as potential biomarkers, to be combined with data from food questionnaires. Moreover, expression levels of *TAS1R3* in PBCs may predict the risk of accumulating excess fat over time, more accurately than the measurement of sugary food consumption.

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