



Nutrición Hospitalaria



Trabajo Original

Obesidad y síndrome metabólico

Comparison of gene expression profile between blood cells and white adipose tissue of patients with obesity

Comparación del perfil de expresión génica entre células de la sangre y el tejido adiposo blanco de pacientes con obesidad

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Abstract

Introduction: Gene expression analyses from peripheral blood mononuclear cells (PBMC) and white adipose tissue are conflicting. It seems that results from single tissue are not enough to explain how changes affect humans as a complex biological system.

Objective: The aim of this study was to compare, from obesity subjects, PBMC and white adipose tissue gene expression that regulates adipogenesis (perilipin 1 [PLIN1], adrenoceptor beta 3 [ADRB3] and peroxisome proliferator-activated receptor [PPARG2]) and the energy metabolism (uncoupling protein UCP1, UCP2 and UCP3) process.

Methods: This study enrolled 35 obese patients, with a body mass index (BMI) > 40 kg/m² (obesity group [OG]), and ten eutrophic health subjects, 18 > BMI > 24.9 kg/m² (control group [CG]). Anthropometric and body composition data were assessed at recruitment using standardized protocols. Samples of peripheral blood and subcutaneous adipose tissue (biopsy) were collected to analyze gene expression by RT-qPCR technique. For statistical analysis, we used the Shapiro-Wilk test and Wilcoxon tests by the SPSS software version 20.0; a p < 0.05 significance level was adopted.

Results: There were significant differences of PLIN1, ADRB3, PPARG2 and UCP3 expression between blood against adipose tissue samples, showing that these genes are upregulated in adipose tissue. UCP2 expression was upregulated in PBMC.

Conclusion: The PLIN1, ADRB3, PPARG2 and UCP3 genes were preferentially expressed in adipose tissue. However, UCP2 was upregulated in PBMC, suggesting that this gene may be assessed in a peripheral blood cell, which is easily accessible, safe and practical.

Key words:

Gene expression.
Adipose tissue.
Blood cells. Obesity.
Real-time polymerase chain reaction.

Resumen

Introducción: la expresión de genes de células mononucleares de sangre periférica y de tejido adiposo blanco es contradictoria. Los resultados del tejido no son suficientes para explicar cómo afectan los cambios al ser humano como un sistema biológico complejo.

Objetivo: el objetivo de este estudio fue comparar, en individuos con obesidad, la expresión de genes que regulan los procesos de adipogénesis (PLIN1, ADRB3 y PPARG2) y el metabolismo energético (UCP1, UCP2 y UCP3) en sangre y tejido adiposo blanco.

Métodos: este estudio incluyó a 35 pacientes con obesidad e índice de masa corporal (IMC) > 40 kg/m² en el grupo obesidad (GO) y a diez personas sanas con peso normal (18 > IMC > 24,9 kg/m²) en el grupo control (GC). Los datos antropométricos y de composición corporal fueron obtenidos por protocolos estandarizados. Se recogieron muestras de sangre periférica y tejido adiposo subcutáneo (biopsia) para analizar la expresión génica por la técnica de RT-qPCR. Para el análisis estadístico se utilizaron el test de Shapiro-Wilk y pruebas de Wilcoxon mediante el programa SPSS versión 20.0 (p < 0,05).

Resultados: no se encontraron diferencias significativas en la expresión de genes PLIN1, ADRB3, PPARG2 y UCP3 entre la sangre y las muestras de tejido adiposo, mostrando que estos genes son regulados positivamente en el tejido adiposo. La expresión del gen UCP2 fue regulada positivamente en sangre.

Conclusión: los genes PLIN1, ADRB3, PPARG2 y UCP3 se expresaron de forma preferente en el tejido adiposo. Sin embargo, el gen UCP2 se reguló positivamente en sangre, lo que sugiere que puede ser evaluado en sangre periférica, que es fácilmente accesible, de forma segura y práctica.

Palabras clave:

Expresión génica.
Tejido adiposo.
Células de la sangre.
Obesidad. Tiempo real de reacción en cadena de polimerasa.

Received: 03/08/2016

Accepted: 14/10/2016

Source(s) of support: São Paulo Research Foundation (FAPESP) (#grant No. 2013/12819-4) and National Council for Scientific and Technological Development (CNPq) (#grant No. 480763/2013-5).

Pinhel MAS, Noronha NY, Nicoletti CF, Quinhoneiro DCG, Oliveira BAP, Cortes-Oliveira C, Salgado-Junior W, Silva-Junior WA, Marchini JS, Souza DRS, Nonino CB. Comparison of gene expression profile between blood cells and white adipose tissue of patients with obesity. Nutr Hosp 2017;34:603-607

DOI: <http://dx.doi.org/10.20960/nh.438>

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INTRODUCTION

High-throughput genomic technologies have provided opportunities for increased understanding of nutritional modulation of gene and proteins expression (1,2). However, this approach has limitations, such as evaluating slight changes and limited access to specific tissues, especially in patient samples composed of healthy volunteers.

According to the literature, some genes are expressed in tissue-specific manner (2,3), consequently, it is necessary to access to the biological material. In cases of patients who will undergo surgery or biopsy, collecting tissue is considered as feasible. Otherwise, without this procedure, collection of internal organ samples such as visceral and adipose tissue is less probable, especially for ethical reasons (4,5).

A minimally invasive method for the study of health-illness transitions is the collection of blood obtained by using the standard venipuncture technique. Peripheral blood mononuclear cells (PBMC) are exposed to all physiological changes, including the change of nutrient concentrations according to their ingestion and absorption (1,6-8).

The evaluation of PBMC for transcriptome analysis has been successfully used to discriminate potential diseases to healthy populations (7-10). Despite PBMC is easily obtainable compared to adipose tissue, liver and muscle, literature has shown conflicting results, and assessing a single tissue could not be enough to fully understand changes in the body (1-3). In this study, we aimed to determine whether targets gene expression in the PBMC is able to reflect the same expression profile in the subcutaneous white adipose tissue in patients with severe obesity.

MATERIALS AND METHODS

This transversal study enrolled patients from a mixed population (11), 35 obese grade III individuals (OG) with a BMI > 40 kg/m² and ten eutrophic individuals (BMI ranging from 18.5 kg/m² to 24.9 kg/m²) as a CG. The sample size was defined by convenience, according to the number of patients treated at the hospital where they were selected. The control individuals underwent surgery of an umbilical hernia (incisional or epigastric) or gallstones without acute cholecystitis, both from the Clinical Hospital of Ribeirão Preto Medical School, of the University of São Paulo. After a thorough introduction to the study, all participants gave their written informed consent to participate. This study was approved by the Ethical Committee of the institution (Certificate of Presentation for Ethical Consideration - CAAE: 18973913.0.0000.5440).

Anthropometric and body composition data, blood and adipose tissue were collected from both groups. Weight (kg), height (m) and BMI (kg/m²) were determined at recruitment using standardized protocols. Body composition was assessed by bioelectrical impedance through Quantum BIA 101 q-RJL Systems analyzer (Clinton Township, MI, USA). Adipose tissue was collected during the surgery procedure or by biopsy in the right upper quadrant region above umbilicus cicatrix.

GENE EXPRESSION

RNA was extracted from PBMC and white adipose tissue for gene expression analysis of gene involved in adipogenesis and energy metabolism (PLIN1, *ADRB3* and *PPARG2*, UCP1, UCP2 and UCP3).

RNA was extracted from samples of subcutaneous white adipose tissue and PBMC using the phenol-chloroform extraction method modified by Chomczynski & Sacchi (1987) (12). The real-time quantitative polymerase chain reaction (RT-qPCR) method was prepared as described above. RNA was reverse transcribed using a high capacity cDNA reverse transcription with a RNase inhibitor kit (Applied Biosystems-Life Technologies, Carlsbad, CA). Quantitative PCR was performed in triplicate on the ABI 7500 Fast Plate (Applied Biosystems). The RT-qPCR reactions were assembled based on the TaqMan Universal PCR Master Mix protocol (Applied Biosystems), using the following genes: UCP1 (Hs00222453_m1), UCP2 (Hs01075227_m1), UCP3 (Hs01106052_m1), *ADRB3* (Hs00609046_m1), PLIN1 (Hs00160173_m1) and *PPARG2* (Hs01115513_m1). *GAPDH* (Hs02758991_m1) and *ACTB* (Hs99999903_m1) were used as the calibrator genes. Quantitative real-time PCR was performed essentially as described and followed the MIQE guidelines (13). The fold change in gene expression in PBMCs and white adipose tissue is quantified relative to the use of the control group as calibrator in this study.

STATISTICAL ANALYSIS

Descriptive statistics consisted of mean values and standard deviation. The normality of data was verified by the Shapiro-Wilk test. The Mann-Whitney test was used to compare gene expression between tissues. Statistical significance was set at $p < 0.05$,

Table I. Anthropometric and body composition data of obese patients and normal weight individuals

Variables	Obese subjects (OG)	Eutrophic subjects (CG)
Number of individuals	35	10
Age (years)	34 ± 8	34 ± 11
Weight (kg)	130 ± 27	57 ± 7 ^a
Height (cm)	163 ± 1	161 ± 7
BMI (kg/m ²)	49 ± 10	22 ± 2 ^a
AC (cm)	133 ± 18	79 ± 9 ^a
LBM (kg)	62 ± 8	40 ± 6 ^a
% LBM	48 ± 6	69 ± 4 ^a
FM (kg)	69 ± 21	17 ± 3 ^a
% FM	52 ± 2	31 ± 4 ^a

Unpaired *t* test. BMI: Body mass index; AC: Abdominal circumference; LBM: Lean body mass; FM: Fat mass. ^a $p < 0.05$ compared to OG.

and all analyses were performed in the Statistical Package software for Social Sciences (SPSS version 20.0 Inc. Chicago. IL).

RESULTS

Anthropometric and body composition data are described in table I. We found, as expected, that weight, BMI, waist circumfer-

ence (WC), lean mass (LM, kg), fat mass (FM, kg) and fat proportion (%) were increased in patients with obesity grade III compared with eutrophic ($p < 0.05$).

Figure 1 shows the relative gene expression in blood and adipose tissue of target genes. The present study indicates that the UCP1 gene was slightly expressed in evaluated tissues, and PLIN1, ADRB3, PPARG2 and UCP3 were upregulated only in samples of subcutaneous adipose tissue in patients with severe

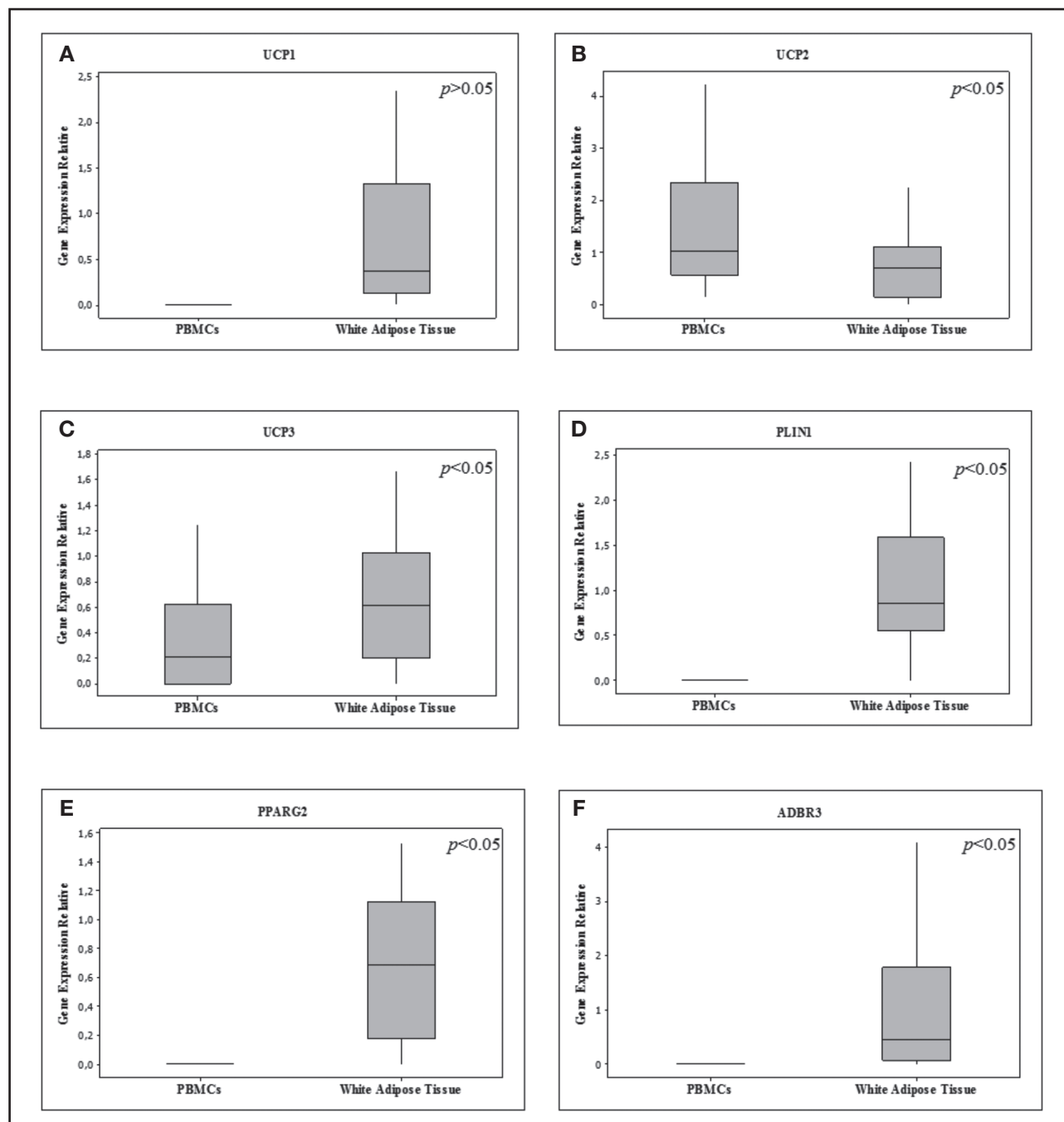


Figure 1. Gene expression in mononuclear cells from peripheral blood (PBMC) and white adipose tissue of obese patients. A. UCP1. B. UCP2. C. UCP3. D. PLIN1. E. PPARG2. F. ADRB3.

obesity. On the other hand, UCP2 expression was upregulated in blood cells in the same patients (Fig. 1A). Statistical analysis showed some outliers, therefore, a new analysis was performed by removing possible confounding factors, but results remained the same. In order to avoid reducing the number of samples, original values, which are featured by asterisk in the graphs, are maintained (Figs. 1A-F).

DISCUSSION

In the current study, we demonstrated that the expression of genes involved in energy and lipid metabolism is different in white subcutaneous adipose tissue when compared to mononuclear cells from peripheral blood of patients with severe obesity. We observed that expression of UCP2 was upregulated in blood samples, whereas UCP3, PLIN1, PPARG2 and ADRB3 were upregulated in white adipose tissue.

The main purpose of this study was to identify similarities between gene expression in PBMCs and white adipose tissue, and validate the use of peripheral blood considering easy access, availability and handling. However, all genes evaluated behaved differently among tissues, suggesting that it is not possible to obtain a reflection of all changes in specific tissues from this biological material.

Dietary interventions for weight loss have shown an impact of several genes on subcutaneous adipose tissue (14-16), although intra- and inter-individual variation has not been fully studied, especially in blood samples. Brattbakk et al. (2013) (3) showed that the correlation between gene expression in peripheral blood and adipose tissue varies according to gene function, suggesting that more studies about this relation are needed for better understanding the impact of diet on the body. In the same way, a recent study showed that the expression of some genes in white adipose tissue and blood can be used for nutritional studies as predictive markers expansion of white adipose tissue (17). Likewise, a study by Diaz-Rua et al. (2015) (18) argues that the analysis of gene expression in PBMCs allows the detection of physiological deviations induced by diet.

Nutrigenomic studies in humans involving the regulation of gene transcription in different tissues are still scarce, first, because this is a relatively new scientific field, and also, because it is not being fully integrated into clinical practice. Moreover, costs for the implementation of this technology are still significant, especially in numerous approaches involving populations (1).

Additionally, most studies in this area aim to identify whether changes in gene transcription can be detected after changes in food intake, suggesting that food and nutrients can cause a significant impact on the ability of adaptive cellular response to changes in the gene expression pattern (1,19). Nevertheless, for this analysis, specific tissue fragments, which are often difficult to access, are needed to evaluate the best response induced by intervention.

The present study found that some genes are more significantly expressed in white adipose tissue; however, the UCP2 gene, an important regulator of energy metabolism, is more expressed in

blood cells. Alternatively, Mello et al. (2012) (2) emphasize that gene expression of PBMC after a dietary intervention can be used to examine the gene response related to the PPAR path and cholesterol metabolism, suggesting that PBMCs appear to be promising for their use in nutritional genomics.

Other studies suggest that gene expression in peripheral blood may be more variable when compared to other tissues (3,20). However, literature has also shown that gene expression of the same individual at the right time is stable in blood cells (7,8,21,22). These factors suggest that peripheral blood could be used in transcriptome analysis studies to verify responses to dietary interventions derived from the same individual over time (3).

The main limitations of this study relate to the sample size and the reduced number of genes evaluated. However, our research group has achieved positive results for the analysis of gene expression in small numbers of patients (16,23), although it would be important to assess the whole transcriptome comparing PBMCs and specific tissue to detect biomarkers for disorders or physiological changes related to the pathogenesis of diseases. Thus, having a gene signature from a biological material easily accessible, such as blood cells, would be important so that this approach could identify specific nutritional problems and chances for disease.

In conclusion, the current study demonstrated that the expression of genes involved in energy expenditure (UCP3) and lipid metabolism (PLIN1, PPARG2 and ADRB3) are downregulated in peripheral blood of patients with severe obesity. Moreover, UCP2 is upregulated in PBMCs, suggesting that the expression of this gene in peripheral blood could be used as a marker present in the material easily accessed and handled.

ACKNOWLEDGMENTS

The authors would like to thank the São Paulo Research Foundation (FAPESP) (#grant no. 2013/12819-4) and the National Council for Scientific and Technological Development (CNPq) (#grant no. 480763/2013-5).

ETHICAL APPROVAL

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the Declaration of Helsinki of 1964 and its later amendments or comparable ethical standards. This study was approved by the Ethics Committee of the institution (Certificate of Presentation for Ethical Consideration - CAAE: 18973913.0.0000.5440).

REFERENCES

1. Afman LA, Muller M. Human nutrigenomics of gene regulation by dietary fatty acids. *Progr Lipid Res* 2012;51(1):63-70.
2. De Mello VD, Kolehmanien M, Schwab U, Pulkkinen L, Uusitupa M. Gene expression of peripheral blood mononuclear cells as a tool in dietary interven-

- tion studies: What do we know so far? *Mol Nutr Food Res* 2012;56(7):1160-72.
3. Brattbakk HR, Arbo I, Aagaard S, Lindseth I, de Soysa AK, Langaas M, et al. Balanced caloric macronutrient composition downregulates immunological gene expression in human blood cells-adipose tissue diverges. *OMICS* 2013;17(1):41-52.
 4. Radonjic M, De Haan JR, Van Erk MJ, Van Dijk KW, Van den Berg SA, De Groot PJ, et al. Genome-wide mRNA expression analysis of hepatic adaptation to high-fat diets reveals switch from an inflammatory to steatotic transcriptional program. *PLoS One* 2009;4(8):e6646.
 5. Centis E, Marzocchi R, Di Domizio S, Ciaravella MF, Marchesini G. The effect of lifestyle changes in non-alcoholic fatty liver disease. *Dig Dis* 2010;28(1):267-73.
 6. Fuchs D, Piller R, Linseisen J, Daniel H, Wenzel U. The human peripheral blood mononuclear cell proteome responds to a dietary flaxseed-intervention and proteins identified suggest a protective effect in atherosclerosis. *Proteomics* 2007;7(18):3278-88.
 7. Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA, et al. Individuality and variation in gene expression patterns in human blood. *Proc Natl Acad Sci USA* 2003;100(4):1896-901.
 8. Radich JP, Mao M, Stepaniants S, Biery M, Castle J, Ward T, et al. Individual-specific variation of gene expression in peripheral blood leukocytes. *Genomics* 2004;83(6):980-8.
 9. Martin KJ, Graner E, Li Y, Price LM, Kritzman BM, Fournier MV, et al. High-sensitivity array analysis of gene expression for the early detection of disseminated breast tumor cells in peripheral blood. *Proc Natl Acad Sci USA* 2001;98(5):2646-51.
 10. Valk PJ, Verhaak RG, Beijnen MA, Erpelinck CA, Barjesteh van Waalwijk van Doorn-Khosrovani S, Boer JM, et al. Prognostically useful gene-expression profiles in acute myeloid leukemia. *N Engl J Med* 2004;350(16):1617-28.
 11. Pena SD, Di Pietro G, Fuchshuber-Moraes M, Genro JP, Hutz MH, Kehdy Fde S, et al. The genomic ancestry of individuals from different geographical regions of Brazil is more uniform than expected. *PLoS One* 2011;6(2):e17063.
 12. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162(1):156-9.
 13. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin Chem* 2009;55(4):611-22.
 14. Capel F, Viguerie N, Vega N, Dejean S, Arner P, Klimcakova E, et al. Contribution of energy restriction and macronutrient composition to changes in adipose tissue gene expression during dietary weight-loss programs in obese women. *J Clin Endocrinol Metab* 2008;93(11):4315-22.
 15. Mangravite LM, Dawson K, Davis RR, Gregg JP, Krauss RM. Fatty acid desaturase regulation in adipose tissue by dietary composition is independent of weight loss and is correlated with the plasma triacylglycerol response. *Am J Clin Nutr* 2007;86(3):759-67.
 16. Oliveira BA, Pinhel MA, Nicoletti CF, Oliveira CC, Quinhoneiro DC, Noronha NY, et al. UCP1 and UCP3 expression is associated with lipid and carbohydrate oxidation and body composition. *PLoS One* 2016;11(3):e0150811.
 17. Voigt A, Ribot J, Sabater AG, Palou A, Bonet ML, Klaus S. Identification of Mest/Peg1 gene expression as a predictive biomarker of adipose tissue expansion sensitive to dietary anti-obesity interventions. *Genes Nutr* 2015;10(5):477.
 18. Diaz-Rua R, Keijer J, Caimari A, Van Schothorst EM, Palou A, Oliver P. Peripheral blood mononuclear cells as a source to detect markers of homeostatic alterations caused by the intake of diets with an unbalanced macronutrient composition. *J Nutr Biochem* 2015;26(4):398-407.
 19. Van Ommen B, Bouwman J, Dragsted LO, Drevon CA, Elliott R, De Groot P, et al. Challenges of molecular nutrition research 6: The nutritional phenotype database to store, share and evaluate nutritional systems biology studies. *Genes Nutr* 2010;5(3):189-203.
 20. Fan H, Hegde PS. The transcriptome in blood: Challenges and solutions for robust expression profiling. *Curr Mol Med* 2005;5(1):3-10.
 21. Eady JJ, Wortley GM, Wormstone YM, Hughes JC, Astley SB, Foxall RJ, et al. Variation in gene expression profiles of peripheral blood mononuclear cells from healthy volunteers. *Physiol Genomics* 2005;22(3):402-11.
 22. Karlovich C, Duchateau-Nguyen G, Johnson A, McLoughlin P, Navarro M, Fleurbaey C, et al. A longitudinal study of gene expression in healthy individuals. *BMC Med Genomics* 2009;2:33.
 23. De Oliveira BA, De Souza Pinhel MA, Nicoletti CF, De Oliveira CC, Quinhoneiro DC, Noronha NY, et al. UCP2 and PLIN1 expression affects the resting metabolic rate and weight loss on obese patients. *Obes Surg* 2016. E-pub ahead of print.