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Distinctive modulation of inflammatory and metabolic parameters in relation to zinc nutritional status in adult overweight/obese subjects☆

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Abstract

Overweight and obesity are associated with low grade of inflammation and chronic inflammatory response characterized by abnormal production and activation of some pro-inflammatory signalling pathways. Taking into account that obesity is the direct result of an imbalance between energy intake and energy expenditure, the nutritional factors in the diet, with particular focus on zinc, may play a pivotal role in the development of obesity-associated comorbidities. Considering the potential interactions among zinc nutritional status, inflammation, overweight/obesity and insulin secretion, the aim of the present work was to clarify the influence of zinc dietary intake on some metabolic, inflammatory and zinc status parameters in adult overweight/obese subjects. We found a close interrelationship between nutritional zinc and obesity. In particular, subjects with a lower zinc dietary intake display a deeper inflammatory status, general impairment of the zinc status, an altered lipid profile and increased insulin production with respect to obese subjects with normal zinc dietary intake. Moreover, in the presence of low dietary zinc intake, the obese subjects are less capable to respond to oxidative stress and to inflammation leading to the development of obesity or to a worsening of already preexisting obesity status. In conclusion, a possible zinc supplementation in obese subjects with a deeper inflammatory status and more altered zinc profile may be suggested in order to limit or reduce the inflammation, taking also into account that zinc supplementation normalizes "inflammaging" as well as zinc profile leading to a correct intra- and extracellular zinc homeostasis.

Keywords: Zinc; Obesity; Inflammation; Array gene expression; Metabolism

1. Introduction

Overweight and obesity have reached epidemic proportions globally and they contribute to the global burden of chronic disease and disability [1]. They are associated with low grade of inflammation and chronic inflammatory response and characterized by abnormal production and activation of some pro-inflammatory signalling pathways, resulting in the induction of several biological markers of inflammation, such as C-reactive protein (CRP), alpha 2-macroglobulin (A2M) and pro-inflammatory cytokines [2]. Conversely, a reduction in body weight is accompanied by a decrease or even a normalization of these biological parameters [3,4]. This association is therefore meaningful because it suggests that these inflammatory processes have a causal relationship with obesity and its comorbidities, such as insulin resistance, type 2 diabetes and cardiovascular diseases [5]. Of relevance, a large number of overweight individuals have a risk of developing obesity and its complications at some later time [6]. In this context, widespread factors, including metabolic and genetic factors, are involved in affecting this association as well as obesity complications [7]. However, taking into account that obesity is the direct result of an imbalance between energy intake and energy expenditure and that an excess of energy stored in adipose tissue in the form of triglycerides may influence appetite and metabolism [8],

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the nutritional factors in the diet may play a pivotal role in the development of obesity. Among them, the trace element zinc may be relevant for the following reasons. First of all, zinc is involved in inflammatory/immune response [9]. Zinc deficiency, as it occurs in chronic inflammation like in type 2 diabetes, leads to a defect in insulin storage and secretion, via a zinc transporter ZnT8 modulation, with subsequent development of insulin resistance and worsening of the inflammatory status [10]. Moreover, a relationship between serum Zn levels and the anabolic and catabolic mechanisms in obesity has been reported [11] as well as reduced plasma levels in obese subjects when compared to normal individuals [12]. Studies in obese animals (ob/ob mice) have further shown an altered zinc metabolism coupled with impaired glucose tolerance, insulin resistance and decreased insulin signalling [13], restored by Zn supplementation [14]. Also, mice fed with low-zinc diet display the same metabolic defects restored by zinc supplementation [15]. In terms of adipose metabolism, one study reports that Zn supplementation may increase total carcass body fat in ob/ob mice and mice fed a high-fat diet (80% fat/20% protein) [16]. Therefore, taken altogether, these observations suggest the existence of potential interactions among zinc nutritional status, inflammation, overweight/obesity and insulin secretion in which the dietary zinc may be considered the pillar. However, the role played by dietary zinc is scarcely studied in the development of obesity. Therefore, the aim of the present work was to clarify the influence of zinc dietary intake on some metabolic, inflammatory and zinc status parameters in adult overweight/obese subjects. The evaluation of the zinc status includes, other than plasma zinc, the measurement of labile pool intracellular Zn as well as intracellular metallothioneins (MTs) and the capacity of zinc release by MT. These last two parameters are fundamental because MTs are the main proteins involved in intracellular zinc homeostasis [17] and in antioxidant activity through the zinc release by MT itself during inflammation and oxidative stress [18]. Taking into account that chronic inflammatory status [2] and oxidative stress [19] are usual and common events in overweight and obese subjects and a limited capacity in zinc release by MT occurs in chronic inflammation [20], such a capacity is also crucial in obesity in order to reduce the inflammation and oxidative stress. The expression patterns of some zinc-dependent genes, in particular the zinc transporter families and genes involved in inflammatory and metabolic mechanisms, have also been studied in peripheral blood mononuclear cells (PBMCs) from obese subjects using a custom microarray. Although adipocytes are used to study inflammation in obesity, PBMCs are herein used taking into account the critical role played by PBMCs in infiltrating adipose tissue with subsequent development of the inflammatory process in obesity [21]. Finally, since obese subjects also show altered psychological parameters [22], which, in turn, are affected by zinc in chronic inflammation [23], some psychological parameters and eating behaviors are also reported in obese subjects in relation to zinc dietary intake. As such, an exhaustive picture on the role played by zinc nutritional status may be outlined in obesity.

2. Materials and methods

2.1. Subjects

A total of 223 overweight/obese individuals (125 females and 98 males) (mean age 43±5 years) with a body mass index (BMI) ≥25 kg/m^2 were enrolled from the Division of Endocrinology, Polytechnic University of Marche (Ancona, Italy) and from the National Institute for Food and Nutrition Research (Rome, Italy). The subjects were then subdivided in two groups: Group 1 (n=100) with low-zinc dietary intake (<7 mg/day for females and <9.5 for males) and Group 2 (n=123) with normal-zinc dietary intake (≥7 mg/day for females and ≥9.5 for males), as reported by RDA for adult subjects [24]. Complete blood analysis and clinical history were obtained from each patient in order to exclude patients with comorbidities. The INRCA Hospital Ethics Committee approved the project and informed consent was obtained from each individual in compliance with Italian legislation and the Helsinki Declaration.

2.2. Collection of blood samples

Fifteen milliliters of peripheral blood was collected in heparinised tubes, and erythrocytes were removed using HAES (Fresenius Kabi Italia Srl, Verona, Italy). Plasma was separated after centrifugation at 2000–3000×g for 10 min at room temperature. PBMCs were obtained by Ficoll-hypaque (density d=1.077 g/ml) (Biochrom AG, Berlin, Germany) gradient centrifugation of heparinised blood.

2.3. Biochemical parameters and alpha-2 macroglobulin determination

Biochemical plasma parameters (see Table 2) were determined with standard laboratory procedures. Plasma alpha-2 macroglobulin was tested using the human alpha-2 macroglobulin ELISA Kit (Assaypro, USA). The minimum detectable level of alpha-2 macroglobulin is typically <1 μg/ml.

2.4. Dietary intake estimation

Dietary intakes of micronutrients, including zinc, were estimated using a semi-quantitative food frequency questionnaire (SFFQ) that each subject filled up. The SFFQ includes 119 food items (the most commonly consumed by the Italian population). The SFFQ was organized in 12 food categories: cereal and cereal products, legumes and pulses, vegetables, fruits, eggs, meat and meat products, fish and fish products, milk and dairy products, sweet, beverages (alcoholic and nonalcoholic), oils and fats, and others. The participants were asked about their usual diet during the last year. For each item on the food list, respondents were asked to estimate the frequency of consumption based on specified frequency categories indicating the number of times the food had usually been consumed per day, week, month or year, and to indicate their typical serving size by checking one of the specific quantities proposed. With the aim to help the respondents indicate the usually consumed portion size, together with the SFFQ a selection of images of typical dishes of the Italian culinary tradition were provided. The questionnaires were then checked by a dietitian. SFFQ data were converted into energy, and macro- and micronutrients by the relevant food composition tables [25]. As is common knowledge, data obtained by SFFQ are less accurate than those obtained by food diaries. To be sure of the quality of collected data, among 60 participants a comparison between data obtained by SFFQ and by 4-day food records was carried out and no significant differences were found (data not shown), suggesting the validity of the data obtained by SFFQ.

2.5. RNA Isolation, target RNA preparation, hybridization and microarray analysis

Microarray analysis was performed in a subset of the total subjects recruited which consisted of 10 individuals per group. This subset reflects the biochemical and nutritional parameters found in the totality of the subjects enrolled.

Total RNA was isolated from frozen PBMC (10×10^6) using the SV Total RNA Isolation System kit (Promega, Madison, WI, USA) and quantified by a spectrophotometer. The quality of the RNA samples was validated by denatured agarose gel electrophoresis according to the integrity of 28S and 18S rRNAs. Gene expression profiling was performed using a custom array (480 oligonucleotides) designed to profile the expression of zinc responsive genes, inflammatory and metabolic pathways (GEArray Custom, SuperArray, USA). Retrotranscription, hybridization and chemiluminescent detection kits were purchased from SuperArray and performed according to the manufacturer's protocol. The images were acquired using the Chemidoc instrument (Biorad, Hercules, CA, USA) and the expression data were generated using the GEArray Expression Analysis Suite Tutorial (SuperArray USA) and the caGEDA data analysis tool [26]. The threshold of differential expression between the two experimental groups of subjects was set at ≥1.5-fold (up-regulation) and ≤0.5-fold (down-regulation) [27].

2.6. Real-time reverse-transcription PCR

RNA was isolated from PBMCs as described before and was reverse transcribed using i-Script reverse transcriptase (Biorad) according to the manufacturer's guidelines. Real-time RT-PCR was performed using the Biorad iCycler Quantitative Thermal Block (Biorad). The RT reaction product (1 μg) was amplified in a 25-μl reaction with iQ SYBR GREEN SUPERMIX (Biorad) for the β-actin housekeeping gene, IL1-β and α-2M genes. Fast Start TaqMan Probe Master (Roche Diagnostics, Indianapolis, IN, USA) was used for the amplification of SLC39A3 and MT1A genes. Probes and primer sequences used for real-time RT-PCR are listed in Table 1. Conditions for amplification were as follows:

β-Actin: 50 cycles of 95°C for 15 s, 62°C for 30 s and 55°C for 50 s.
α-2M: 45 cycles of 95°C for 15 s, 60°C for 30 s and 55°C for 50 s.
IL1-β: 45 cycles of 95°C for 15 s, 60°C for 30 s and 55°C for 50 s.
SLC39A3: 95°C for 10 min followed by 45 cycles of 95°C for 15 s and 60°C for 30 s.
MT1A: 95°C for 10 min followed by 45 cycles of 95°C for 15 s and 60°C for 30 s.

All samples were run in triplicate with both primer sets, and fold changes were calculated using the ΔΔCt method.

Table 1
Taqman probes and primer sequence used for real time RT-PCR

Gene	Probe	Primer
β-Actin		5′-GGATAGCACAGCCTGGATAG-3′
		5′-GCGAGAAGATGACCCAGATC-3′.
α2M		5′-ACCTTTGGGGAGCAGATATG-3′
		5′-TCAGAACAAAGGCTGTGAGC-3′
IL-1β		5′-CTGTCCTGCGTGTTGAAAGA-3′
		5′-TTGGGTAATTTTTGGGATCTACA-3′
MT1A	Universal ProbeLibrary probe #18	5′-ATGCAACTCCTGCAAGAAGAG-3′
	(Roche Applied Science)	5′-GCACACTTGGCACAGCTC-3′
SLC39A3	Universal ProbeLibrary probe #74	5′-GTTTCTGGCCACGTGCTT-3′
	(Roche Applied Science)	5′-AGGCTCAGGACCTTCTGGA-3′

2.7. Flow cytometric analysis of intracellular labile zinc and NO-induced release of zinc from MT

"Zinc-free" RPMI medium was obtained by treatment of RPMI with 5% Chelex 100 (Sigma-Aldrich, Milan, Italy). Thawed PBMCs were divided into two equal aliquots of 2×10^5 cells, at least. One aliquot was incubated with 20 M Zinpyr-1 (ZP-1) (Neurobiotex, Galveston, TX, USA) for 30 min at 37°C, 5% CO_2 in HEPES buffered zinc-free RPMI medium containing 1 mM EDTA, as extracellular chelator, of free zinc eventually still present in the medium and/or adsorbed to the cell membrane. The second aliquot was always incubated in the same conditions plus 50 μM *N*,*N*0,*N*0-tetrakis (2-pyridylmethyl) ethylenediamine (Sigma-Aldrich, Milan, Italy), in order to detect the autofluorescence of the zinc-free ZP-1 probe. After incubation, the aliquots were immediately analyzed by flow cytometry (Coulter Epics XL). After selecting the lymphocyte population according to the forward light and side scatters, the mean fluorescence intensity (MFI) for ZP-1 was detected (excitation wavelength 488 nm and detection at 525±15) in the two aliquots. Data were reported as the ratio of ZP-1 fluorescence/ZP-1 autofluorescence and represented the intracellular labile Zn (iZnL) [28]. To investigate the NO-induced release of Zn, another aliquot was incubated with 20 μM ZP-1 plus 100 μM diethylamine NOnoate acetoxymethylated (AcOM DEA/NO) (Calbiochem, VWR International, Milan, Italy) [29]. In fact, AcOM-DEA/NO is a cell-permeable acetoxymethylated diazeniumdiolate compound that donates NO "intracellularly" following the action of intracellular esterases [30]. Once the incubation period was terminated, all aliquots were immediately read by the flow cytometer. The difference between iZnL in the presence and absence of the NO donor was used to estimate the intracellular release of Zn (iZnR), as previously reported [28].

2.8. Metallothionein detection

Thawed PBMCs (2×10^5) were treated with 0.3% paraformaldehyde and stored at 4°C for 2 days before processing. MT determination was performed as previously reported by Yurkow and Makhijani [31] using the monoclonal mouse antihorse metallothionein clone E9 antibody (Dakocytomation, Denmark). Results were expressed as MFI [32], which was converted into picomole per milligrams of protein (pmol/mg protein) using a calibration curve obtained by incubating PBMCs with different *in vitro* doses of zinc (range 0–100 μM) [33]. Intracellular protein content was calculated using the Quant-it Protein Assay Kit (Invitrogen, Eugene, OR, USA).

2.9. Plasma zinc and total intracellular zinc assay

Total plasma Zn was determined by ICP-MS after the dilution (1:10) of the samples with a solution containing 0.1% HNO_3, 0.15% TRITON X100 (Eastman Kodak Company, Rochester, NY, USA) and 10 ppb of rhodium solution (Merck, Darmstad, Germany) as internal standard.

Total intracellular zinc was also tested in lysate cells using HPLC-ICP-MS with the method previously reported [33].

2.10. Evaluation of psychopathologies and disorders in eating behaviors

To evaluate the presence of psychopathology, the Symptom Check List-90 (SCL-90) [34] questionnaire was administered; values of SCL-90 General Symptomatic Index (GSI) ≥1 were considered indicative of psychopathology. The Binge Eating Disorder (BES) scale [35] was used to evaluate disorders in eating behaviors. The BES diagnosis is considered much probable if the complex score is >27, possible if the score is between 17 and 27, and improbable if the score is <17.

2.11. Statistical analysis

Statistical analysis was performed using the SPSS software (version 11.5, SPSS Inc. Chicago, IL, USA). Univariate analysis was performed to analyze the differences between the two groups studied using as covariates the caloric intake and BMI values.

Table 2
Clinical, metabolic, psychological and nutritional parameters in overweight/obese subjects subdivided on the basis of low (<7 mg/day for females and <9.5 for males) (Group 1) and normal (≥7 mg/day for females and ≥9.5 for males) (Group 2) zinc dietary intake

	Group 1 (n=100)	Group 2 (n=123)
Zn dietary intake (mg/day)	5.66±1.98*	12.20±2.32
Alpha2macroglobulin (μg/ml)	17.84±4.16*	12.11±3.67
C Reactive protein (mg/dl)	0.80±0.19*	0.20±0.07
Glucose (mg/dl)	94.88±10.86	92.14±10.53
Total cholesterol (mg/dl)	245.32±15.58*	204.59±15.88
HDL Cholesterol (mg/dl)	60.24±8.69*	73.63±9.13
LDL Cholesterol (mg/dl)	148.79±18.29*	117.42±12.57
Triglycerides (mg/dl)	156.36±16.21*	88.42±15.24
Insulin (μIU/ml)	10.11±2.06*	8.58±1.26
SLC-90 GSI	0.80±0.36	0.72±0.34
BES	8.29±4.92	7.62±3.67

Values are shown as mean±S.D. Mean values were adjusted using caloric intake and BMI as covariates.
* P<.05 as compared with Group 2 subjects.

Comparison between Zn dietary intake and some of the other parameters evaluated was performed using partial correlation coefficients after controlling for BMI. Results were considered significantly different when P<.05.

3. Results

3.1. Clinical, metabolic, psychological and nutritional analysis

In all subjects, divided between those with low-Zn dietary intake (Group 1) and those with normal-Zn dietary intake (Group 2), some clinical, metabolic, psychological and nutritional parameters (such as Zn dietary intake, BMI, A2M, CRP, glucose, total cholesterol, HDL and LDL cholesterol, triglycerides, insulin, and SLC-90 GSI and BES test results) were evaluated. As shown in Table 2, the subjects in Group 1 showed higher values of inflammatory markers (A2M and CRP) and lipid asset (total and LDL cholesterol, triglycerides), but lower HDL cholesterol values and higher insulin concentration than those in Group 2 (P<.05). By contrast, psychological (SLC-90 GSI and BES tests) parameters were similar between the two groups (Table 2). Of note, the differences between the two groups were not statistically significant with regard to glucose value, suggesting the absence of comorbidity by diabetes mellitus in our recruited subjects.

3.2. Zinc status and MT analysis

Concerning the zinc status, the plasma zinc, intracellular zinc content (iZn), intracellular metallothionein levels, intracellular labile zinc (iZnL) and quota of zinc released by MT (iZnR) were significantly lower in Group 1 than in Group 2 (P<.05) (Table 3).

3.3. Gene expression profiling

Table 4 shows genes differently regulated between the two experimental groups on the basis of the corresponding expression

Table 3
Zinc status parameters in Group 1 and Group 2 subjects

	Group 1 (n=100)	Group 2 (n=123)
Plasma Zn (μM)	12.58±1.37*	13.18±1.30
iZn (nmol/mg protein)	0.40±0.12*	0.59±0.30
MTs (pmol/mg protein)	19.49±3.32*	29.33±4.60
iZnL	1.30±0.11*	1.56±0.15
iZnR	0.15±0.29*	0.26±0.03

Values are shown as mean±S.D. Mean values were adjusted using caloric intake and BMI as covariates.
* P<.05 as compared with Group 2 subjects.

Table 4
List of genes differentially expressed in the two groups of subjects

Gene symbol	Gene name	Accession[a] number	Fold[b] change
I. Zn homeostasis			
SLC30A1 (ZNT1)	Solute carrier family 30 member 1	NM_021194	0.2
SLC39A1 (ZIP1)	Solute carrier family 39 member 1	NM_014437	1.7
SLC39A3 (ZIP3)	Solute carrier family 39 member 3	NM_144564	1.6
MT1A	Metallothionein 1A	NM_005946	0.2
MTF1	Metal-regulatory transcription factor 1	NM_005955	0.4
II. Inflammatory response			
IL6	Interleukin 6	NM_000600	1.6
IL1α	Interleukin 1, alpha	NM_000575	2.5
IL1β	Interleukin 1, beta	NM_000576	1.6
A2M	Alpha-2-macroglobulin	NM_000014	3.5
III. Metabolism modulation			
PPARG	Peroxisome proliferative activated receptor, gamma	NM_015869	0.5
IDE	Insulin-degrading enzyme	NM_004969	0.5
INS	Insulin	NM_000207	1.8
IV. Other genes			
LEP	Leptin	NM_000230	0.4
SIRT1	Sirtuin 1	NM_012238	0.49

[a] Corresponds to the gene sequence from GenBank.
[b] Fold change in gene expression level was calculated as the ratio of average values from Group 1 subjects to Group2 subjects.

fold changes and significance levels ($P<.05$). These genes are implicated in Zn homeostasis, inflammatory response, lipid and glucidic metabolism. Genes involved in Zn homeostasis (SLC30A1, MT1A and MTF1) were down-regulated in Group 1 vs. Group 2, while the zinc importers SLC39A1 and SLC39A3 were up-regulated (Table 4). All the genes involved in the inflammatory response (IL6, IL1-α, IL1-β and A2M) were up-regulated in patients of Group 1 vs. those of Group 2. Among the genes involved in the regulation of lipid and glucidic metabolism, INS was up-regulated, while PPARG and insulin degrading enzyme (IDE) were down-regulated in Group 1 vs. Group 2. Moreover, other genes such as leptin (LEP) and sirtuin (SIRT1) were differentially expressed in the two groups of subjects. To confirm the gene expression changes observed by microarray studies, RT-PCR analysis was performed on a selection of genes (IL1-β, A2M, MT1A and SLC39A3) in a larger number of obese subjects (50 subjects/group) (Table 5)

3.4. Bivariate correlation analysis

In the bivariate correlation analysis, the influence of variables involved in Zn homeostasis, inflammatory response, lipid and glucidic metabolism, and other parameters on Zn dietary intake was evaluated and reported in Table 6. Positive significant correlations have been found between Zn dietary intake and plasma Zn ($r=0.45$), iZn ($r=0.46$), MTs ($r=0.54$), iZnL ($r=0.52$), iZnR ($r=0.63$), SLC30A1 ($r=0.89$), MT1A ($r=0.56$), MTF1 ($r=0.49$), HDL cholesterol ($r=0.49$), PPARG ($r=0.91$), IDE ($r=0.85$), LEP ($r=0.77$) and SIRT1 ($r=0.78$). By contrast, negative correlations have been found between Zn dietary intake and SLC39A1 ($r=-0.45$), SLC39A3 ($r=-0.83$), total cholesterol ($r=-0.47$), LDL cholesterol ($r=-0.50$), triglycerides ($r=-0.51$), A2M protein ($r=-0.58$), CRP ($r=-0.63$), IL6 ($r=-0.65$), IL1-α ($r=-0.67$), IL1-β ($r=-0.87$) and A2M gene ($r=-0.68$).

4. Discussion

Close interrelationships exist among nutritional zinc, zinc status inflammatory condition and metabolism in obesity. In particular, subjects with a lower zinc dietary intake display a deeper inflammatory status, a general impairment of the zinc status (low plasma zinc, decreased intracellular zinc content and reduced intracellular labile zinc), an altered lipid profile and increased insulin production compared to obese subjects with normal zinc dietary intake. Of great interest, subjects with low zinc dietary intake display reduced MT production but, at the same time, a very limited capacity in zinc release by MT itself, suggesting that, in the presence of low dietary zinc intake, the obese subjects are less capable of responding to oxidative stress and to inflammatory status. Such a condition may lead to the development of obesity or at least to a worsening of already preexisting obesity status [12]. Such an assumption is supported by the discovery that zinc is involved in fighting oxidative stress and inflammation, conferring biological activity of some zinc-dependent antioxidant enzymes and balancing the Th1/Th2 paradigm, which, in turn, is unbalanced in chronic inflammation and in obesity towards the induction of Th2 pro-inflammatory profile [36]. On the other hand, a body of evidences suggests the presence of an overall, low-grade inflammation in obesity, with altered levels of several circulating factors such as increased plasma levels of CRP, IL-6 and other biological markers of inflammation [2]. In agreement with these studies, CRP, A2M, PPAR-gamma and the gene expression of some pro-inflammatory cytokines increase especially in obese subjects with low zinc dietary intake. These findings clearly suggest the presence of a close interrelationship between zinc and inflammatory condition in obesity with a particular focus on the zinc content in the diet. Therefore, a good intracellular zinc homeostasis is fundamental in maintaining some body homeostatic mechanisms, including the inflammatory/immune response. Various proteins contribute to the maintenance of intracellular zinc homeostasis. Among them, MT and zinc transporters play a key role. MTs are relevant because of their capacity to sequester and release zinc in presence of oxidative state to some zinc-dependent antioxidant enzymes in order to fight oxidative stress [37]. Zinc transporters [the ZnTs (solute-linked carrier 30

Table 5
Validation of array results by real-time RT-PCR

Gene	Group 1	Group 2
IL1-β	1.41±0.11 *	0.51±0.10
MT1A	0.52±0.13 *	0.75±0.26
A2M	2.31±0.66 *	1.30±0.12
SLC39A3	1.07±0.12 *	0.45±0.09

Values are shown as mean experimental values±S.D.
* $P<0.01$ as compared with obese subjects with normal Zn dietary intake.

Table 6
Partial correlations between Zn dietary intake and some variables involved in Zn homeostasis, inflammatory response, lipid and glucidic metabolism, and other parameters

Variables of Zn homeostasis	Correlation coefficient (r)	P value	Variables of inflammatory response	Correlation coefficient (r)	P value
Plasma Zn	0.45	.05	A2M (μg/ml)	−0.58	.009
iZn	0.46	.044	CRP	−0.63	.007
MTs	0.54	.018	IL6	−0.65	.003
iZnL	0.52	.022	IL1α	−0.67	.002
iZnR	0.63	.004	IL1β	−0.87	.0001
SLC30A1	0.89	.0001	A2M (gene)	−0.68	.001
SLC39A1	−0.45	.05			
SLC39A3	−0.83	.0001			
MT1A	0.56	.013			
MTF1	0.49	.035			

Variables of metabolism modulation	Correlation coefficient (r)	P value	Other variables	Correlation coefficient (r)	P value
Total cholesterol	−0.47	.042	LEP	0.77	.0001
HDL cholesterol	0.49	.03	SIRT1	0.78	.0001
LDL cholesterol	−0.50	.03			
Triglycerides	−0.51	.024			
PPARG	0.91	.0001			
IDE	0.85	.0001			

Mean values were adjusted using BMI as covariate.

family) and ZiPs (solute-linked carrier 39 family)] are relevant because they are deputed to transport zinc ions out (ZnT family) or inside (Zip family) the cell [38]. In such a way, zinc is equally distributed between intra- and extracellular spaces. Chronic inflammation is characterized by zinc deficiency, and its distribution is altered with high expression of the Zip family and low expression of the ZnT family [39,40]. This phenomenon is largely linked to MT expression and production and to their capacity to release zinc in the cytoplasm [17], which, in turn, is strongly limited in chronic inflammation leading to reduced content of intracellular free zinc ions. As such, an altered zinc transporter profile occurs in order to compensate for the intracellular zinc deficiency [41]. Such an alteration of the zinc transporter profile associated with a very limited zinc release by MT is observed in obese subjects with low-zinc dietary intake (Table 3). Taking into account the presence of chronic inflammation in obesity and the pivotal role played by zinc in reducing the inflammatory status through a balance of the Th1/Th2 paradigm [36], it is evident from the findings obtained in the present study that a lower zinc dietary intake is crucial in the development of obesity or in the worsening of preexisting obesity status. The presence of significant correlations between the zinc dietary intake and the main biochemical parameters related to the obesity condition (lipid asset, inflammatory parameters and zinc status profile) is in line with this interpretation. On the other hand, zinc is also involved in the regulation of leptin [42], which is an important component in the neuroendocrine transmission line that regulates appetite and energy balance through neuropeptide Y mRNA level reduction, inhibiting feeding behavior [43]. It has been reported that leptin decreases zinc deficiency coupled with a dysregulation of some cytokines (IL-1, TNF-alpha, IL-2) and insulin [42,44] restored by zinc supplementation [42]. Taking into account that these cytokines regulate the appetite and are involved in obesity and that zinc affects their expressions [39] as well as insulin production in the cellular vesicles [10], the presence of low gene expression of leptin and increased gene expression of insulin coupled with decreased IDE in obese subjects with low-zinc dietary intake is a clear index of the presence of vicious cycle among zinc status, inflammatory condition, leptin and insulin levels in obesity. In this context, zinc status is the core of a possible increase of the obesity condition especially in subjects with incorrect zinc dietary habits leading to insulin resistance and the possible appearance of diabetes type 2 at some later time. On the other hand, IDE belongs to the M16 family of zinc metalloproteases and controls the insulin levels [45]; loss of function of IDE leads to elevated insulin levels and insulin resistance [46], as it occurs in type 2 diabetes which is also characterized by zinc deficiency [47]. Therefore, lack of zinc may promote insulin resistance and chronic inflammation. In this context, taking into account that some pro-inflammatory cytokines antagonize the synthesis of PPAR-gamma and contribute to insulin resistance [48], of relevance is the role played by PPAR-gamma agonists in promoting insulin sensitivity and suppressing the inflammatory process in obesity [44]. Moreover, the existence of a negative strict link between PPAR-gamma and sirtuin proteins [49], which, in turn, have an anti-inflammatory role [50], and the discovery that SIRT-1 is less expressed in obese subjects with low zinc dietary intake (Table 4) further suggest the presence of insulin resistance in obesity that may be mediated by zinc ion bioavailability. The recent discovery of the influence of zinc in the gene expression of PPAR-gamma [51,52] confirmed also in the present study, and the fact that sirtuin 1 utilizes an active site zinc for its deacetylase function [53], may be in line with this interpretation where, however, the zinc status has an irrelevant role on the psychological parameters in our groups of subjects. In conclusion, a more altered zinc profile is present in obese subjects with a deeper inflammatory status (Group 1) with respect to the other ones (Group 2) (Tables 2 and 3). While for the obese subjects belonging to Group 2 a correct zinc dietary intake may be sufficient, zinc supplementation may be suggested in obese subjects belonging to Group 1 in order to limit or reduce the inflammation. Such a proposal is supported by the discovery that zinc supplementation reduces high TNF-α and IL-6 production in the condition of acute and chronic inflammation, such as in infected mice [54], in prematurely aging mice [55] as well as in old humans [56] and in old infected subjects [57]. This anti-inflammatory effect of zinc has also been confirmed by *in vitro* studies, such as in PBMCs stimulated with LPS, in which zinc inhibits lipopolysaccharide-induced TNF-α and IL-1β release [58]. Moreover, taking into account the presence of an altered modulation of the zinc transporters in obese subjects with a deeper inflammation (Table 4) and since zinc also normalizes zinc transporter profiles with subsequent more correct intra- and extracellular zinc homeostasis [59], zinc supplementation in obese subjects of Group 1, actually in progress in our laboratory, may be useful.

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