

Diets containing dairy foods positively affects weight and fat loss and cytokines blood levels in premenopausal obese women

Filippo Rossi^{a,*}, Battezzati Alberto^b, Capraro Jessica^{a,1} and Bertoli Simona^b

^a*Istituto di Scienze degli alimenti e della Nutrizione, Facoltà di Scienze Agrarie, Alimentari ed Ambientali, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, Piacenza, Italy*

^b*Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Sezione di Nutrizione Umana, Facoltà di Scienze Agrarie e Alimentari, Università degli Studi, Via Colombo 60, Milano, Italy*

Abstract.

BACKGROUND: Several researches studied the relationship between Ca assumption and overweight/obesity. Levels of Ca supplementation higher than requirement, were related to increased fat loss in subjects at ipo-caloric diet and Ca from dairy foods resulted more effective than Ca from mineral source in the promotion of weight loss.

OBJECTIVE: Since the available studies were conducted using very high levels of Ca supplementation or on subjects fed Ca lacking diet, we studied the role of Ca from dairy foods on subjects receiving ipo-caloric diet, with Ca content adequate to the requirements.

METHODOLOGY: 40 pre-menopausal, women nursed for their obesity and eating or not dairy foods, were recruited for the study. They received ipo-caloric diets, with Ca equal to requirements. Subjects avoiding dairy products received also a Ca supplement (No Dairy group, $n = 15$), while other women eating at least 2 servings/d of low fat dairy products (Dairy group, $n = 40$). Body weight, abdominal fat and blood parameters were monitored at the beginning and at the end (3 months) of the experiment. Age and BMI were: 37.7 ± 7.5 years and 34.4 ± 3.7 for subjects included in the Dairy group and 39.8 ± 9.8 years and 33.8 ± 3.5 for women of No Dairy group.

RESULTS: Women in the Dairy group showed a weight loss of 7.03% respect the initial weight, while in women avoiding dairy products the weight loss was 3.21% ($P < 0.01$). The whole body fat loss was 10.79% and 6.0%, for Dairy and No Dairy respectively, however the consumption of Ca from dairy foods did not affect waist circumference and abdominal-visceral fat.

No evidence of significant effect of the food treatments on the main haematological parameters related to the adipose tissue metabolism were seen. Insulin levels did not showed significant variations. Leptin concentration in blood decreased between the beginning and the conclusion of the trial as a consequence of fat mass decrease ($P < 0.10$), but without difference between the dietary treatments. Instead the IL-6 showed a higher reduction in subjects receiving the Dairy⁺ diet compared to diet without dairy products. The IL-6 levels showed a significant decrease between the beginning and the end of the experiment, apart from the diet (1.20 vs 0.66 pg/ml; $P 0.0135$).

Both the adiponectin and the unesterified fatty-acid concentration were not influenced by the diet but, while adiponectin decreased between the beginning and the end of the study, NEFA did not show significant variations along time.

¹ Present address: Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Sezione di Scienze Chimiche e Biomolecolari, Facoltà di Scienze Agrarie e Alimentari, Università degli Studi di Milano, Via Celoria 2, Milano, Italy.

*Corresponding author: Filippo Rossi, Istituto di Scienze degli alimenti e della Nutrizione, Facoltà di Scienze Agrarie, Alimentari ed Ambientali, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy. Tel.: +39 052 359 9286; Fax: +39 052 359 9259; E-mail: filippo.rossi@unicatt.it.

IL-6 was positively correlated with leptin and fat mass, confirming the relationship between fat accumulation and inflammatory status.

CONCLUSIONS: Diets containing dairy foods have increased body weight and fat loss compared to diet lacking of milk and derivatives. Also the blood levels of IL-6 resulted lower in Dairy diets.

Keywords: Ca, dairy products, IL-6, adiponectin, obesity

1. Introduction

In the last two decades, obesity has been subjected to a rapid growth in Europe [1], so that it can be considered a real epidemic disease. Berghofer et al. [2], in a perspective study, analyzed epidemiologic data from 1980 to 2005, referring to subjects between the ages from 25 to 65, and showed that in Europe the prevalence of obesity in men ranged from 4.0% to 28.3% and in women from 6.2% to 36.5%. At the beginning of years '80, McCarron et al. [3] and Trevisan et al. [4] noted a relationship between Ca assumption and body weight, and Zemel et al. [5], began various studies about the lipolytic activity of intracellular Ca in the adipose tissue. The intracellular calcium $[Ca^{++}]_i$ plays a key role in the adipocyte metabolism regulation, with the mediation of calcitropic hormones: the decrease of parathyroid hormone and vitamin D₃, caused by the dietary uptake of Ca^{++} , stimulates the rapid reduction of $[Ca^{++}]_i$ and this facilitates the repartition of dietary energy from lipid storage to lipid oxidation, by stimulating lipolysis and thermogenesis and inhibiting lipogenesis [6]. It was shown that the dietary calcium can influence metabolism both under caloric restriction, by increasing fat breakdown and accelerating fat loss and also during high energy diets, by attenuating body fat accumulation [6, 7]. Calcium from dairy foods increases the fat oxidation rate [8] although a similar effect between organic or inorganic Ca was also reported [9].

At present it is not clear the effect of dietary Ca on lipolysis, although several studies have been carried out in humans, they were conducted using very high levels of Ca supplements (range 1250–2500 mg/die), or using Ca intake lower than maintenance level.

In our work we evaluate, in pre-menopausal obese women, the effects of low-energy diets with Ca levels equal to the requirements but coming from two different sources, that are low-fat dairy foods or mineral supplements. The effects on body energy balance, glucose/lipid metabolism and body inflammation status were evaluated by measuring the following parameters: body weight, body fat mass and visceral fat loss rate, adipokines (leptin, adiponectin) and inflammatory markers, such as IL-6, insulin and C-peptide blood levels.

2. Material and methods

2.1. Subjects

The experimentation was performed at the International Center for the Assessment of Nutritional Status (ICANS, Milano, Italy). *Subjects were selected between pre-menopausal women with body mass index above cut off for obesity ($>30\text{ kg/m}^2$) seeking weigh loss program at ICANS from July to October 2010.* Two groups of obese women were set up according to their acceptance of dairy foods: women avoiding milk and derivatives were included in the No Dairy Group (No Dairy), while women eating dairy foods formed the Dairy Group (Dairy). Subjects were excluded if they had any disease causing significant nutritional status impairment (Crohn's disease, neoplasia, end-stage renal failure, cirrhosis, congestive heart failure, chronic infection); endocrine disease, including hyper-hypothyroidism, diabetes mellitus, or use of hormones or medication that may affect endocrine function within the previous 2 months; recent (within 1 months) acute illness or injury; weight loss or gain ($>5\text{ kg}$) in the last year; treatment with special diets. *Out of 47 eligible subjects, 7 subjects did not complete one or more of the instrumental examinations so that the present analysis was performed on the remaining 40 subjects.* At baseline, women underwent the following evaluations: family and clinical history, food habits, life style, physical examination; body composition assessment; resting energy expenditure evaluation; blood sample for biochemical parameter measurement. After 3 months the clinical and nutritional assessment was repeated. The study was carried out according to the principles of the Helsinki

Declaration and the protocol was approved by the University of Milan Ethics Committee (Milan, Italy) and a consent form was signed by all subjects before enrolment.

2.2. Anthropometric measurements

The body weight (BW, Kg), body height (HT, cm) and waist measurement (cm) were measured to the nearest 100 g and 0.5 cm respectively. Body mass index (BMI) was defined as the ratio of weight to height squared (kg/m^{-2}). The fat mass (FM) were estimated by plicometry. The skinfold thicknesses were measured in triplicate by means of a Holtain LTD caliper [10]. Fat free mass (FFM) was performed by Human-IM DIP (DS Medigroup, Milan, Italy), software version Human-IM Plus Release 3.0. Briefly the instrument estimates the voltage drop of an applied alternating current at frequencies of 5, 10, 50, 100, 300 Hz. Analysis time was 10 min. Published equations were used to calculate total body water (TBW), extracellular water (ECW), intracellular water (ICW) [11], FFM and FM [12].

Abdominal Subcutaneous fat (SAT) and Abdominal visceral fat (VAT) were measured on fasting patients by the same operator using a Logiq 3 Pro Ultrasonography equipped with a 3.5 MHz convex-array probe and with a 7.5 MHz linear probe (GE Healthcare, Milwaukee, WI, USA) according to a validated standardized protocol [13]. Specifically, SAT was measured as the distance between the epidermis and the external face of the rectus abdominis muscle and VAT was measured as the distance between the anterior wall of the aorta and the posterior surface of the rectus abdominis muscle measured at the level of the xiphoumbilical line or linea alba. The within-day intra-operator coefficient of variation for repeated measures of VAT and SAT in our laboratory is 0.8%.

2.3. Resting energy expenditure measurement

The Resting energy expenditure (REE) was measured by indirect calorimetry with open circuit (Sensor Medics 31, Anaheim, CA, USA). All measurements were made in a thermoneutral environment (24–26°C) and with no external stimulation. Approximately 30 min of respiratory gas exchange data were collected. The first 5–10 min of data were discarded, as recommended by Isbell et al. [14]. This allowed the subjects enough time to adapt herself to the canopy and instrument noise. The average of the last 20 min of measurements was used to determine the daily REE according to standard abbreviated Weir equation [15]: $\text{REE (Kcal/day)} = [3.941 \text{ VO}_2 \text{ (mL/min)} + 1.106 \text{ VCO}_2 \text{ (mL/min)}] * 1.44$.

2.4. Biochemical parameters

Venous blood samples were obtained in the early morning after an overnight fast. Serum was stored as aliquots at -80°C .

Leptin was measured by using a commercial kit for human leptin (Linco Research Inc, Missouri, USA), based on a competitive radio-immuno assay (RIA), using labelled leptin (^{125}I -leptin) and anti-leptin antibodies.

Insulin was detected by using the Elisa technique.

The HOMA index was calculated using the formula: $\text{insulin } (\mu\text{U}/\text{m}) * [\text{glucose (mmol/l)}/22.5]$ [16].

Interleukin 6 was measured by electrochemiluminescence immunoassay (Cobas e411 Hitachi, Roche Diagnostics, Mannheim). Interassay precision (CV) was 3.1%.

Adiponectin (mg/mL) was measured using an enzymatic immunoassay kit (R & D Systems; Wiesbaden, Germany), interassay precision (CV) was 3.4%.

Unesterified fatty-acids were measured by colorimetric assay using an enzymatic kit (cat n° 11383175, Roche Diagnostics, Germany).

Serum glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, were determined by autoanalyzer (Cobas 286 Integra 400 plus, Roche Diagnostics, Mannheim, Germany).

2.5. Diets

The volunteers received two balanced hypocaloric diets (Table 2), formulated using the software WinFood 2.5 and containing 800 mg/die Ca by dairy products (Dairy) or by mineral supplements (No Dairy). Dairy products were

introduced in at least two moments of the day: beyond the breakfast (milk or yogurt), the consumption of yogurt, ricotta or low fat cheeses was indicated.

In both diet the recommended daily Ca intake was achieved by means of other natural Ca sources, like water and vegetable. In order to avoid strong differences in Ca intake from water, the enrolled women were instructed to avoid mineral water with high Ca content.

2.6. Statistics

All statistical analysis were performed using SAS 9.2 for Windows software (SAS Institute, Cary, NC, USA). The difference between diets were evaluated using the Student's *t*-test (PROC GLM). Relationships between blood parameters were evaluated by Pearson's correlation coefficient (PROC REG).

3. Results

3.1. Calcium effects on weight loss and body fat

The clinical trial was performed on 40 premenopausal women, 22 of which completed the experimentation, 13 belonging to the dairy products diet group and 9 to the no-dairy products ones, lasted 95 and 96 days respectively for the two groups. The characteristics of the subjects at the time of enrolment are shown in Table 1.

Before the beginning of the diet, all subjects had a BMI higher than 30, an average waistline circumference of 103 and an average body fat percentage of 47%. After the energetic restriction period, it was observed a weight loss of 7,03% respect the initial weight in the group fed dairy products and 3,21% in the other group (Table 3). Beside, the fat loss respect the initial body fat was 10.79% and 6,0%, respectively (Table 4).

Table 1
Anthropometric parameters of the subjects involved in the clinical trial

	Dairy	No Dairy	P-value
N° of subjects	25	15	
Age (years)	37.7 ± 7.5	39.8 ± 9.8	0.4460
Weight (kg)	88.6 ± 10.8	88.1 ± 9.1	0.8679
BMI	34.4 ± 3.7	33.8 ± 3.5	0.6336
Waist circumference (cm)	103.4 ± 6.7	103.2 ± 10.1	0.9343
Body fat (%)	47.4 ± 4.0	47.0 ± 3.8	0.5665
BMR (kcal/day)	1601 ± 85.3	1612 ± 73.2	0.6801
Blood parameters			
Glucose (mg/dL)	89.0 ± 5.22	92.6 ± 8.88	0.4326
HOMA index	2.24 ± 1.44	2.54 ± 1.47	0.5226
Triglycerides (mg/dL)	97.1 ± 46.3	105.6 ± 47.5	0.5940
Cholesterol (mg/dL)	191.4 ± 34.5	210.6 ± 33.6	0.1020
HDL cholesterol (mg/dL)	53.9 ± 8.9	59.9 ± 15.8	0.1834
LDL cholesterol (mg/dL)	121.5 ± 40.7	130.4 ± 31.6	0.6677

Table 2
Energy, proteins and calcium intake of the two diet types

DIET	kcal	Proteins (g/kg BW)	Ca (mg/die)
Dairy	1388 ± 193	0.86 ± 0.1	880.3 ± 22.8
No dairy	1372 ± 153	0.83 ± 0.1	815.21 ± 16.9

Table 3

Weight loss in obese women fed differently Ca source hypocaloric diets. The results are expressed as mean±standard error

DIET	Weight loss (g/week)	Global weight loss (% initial weight)
Dairy	491.1 ± 47.6	7.03 ± 0.71
No dairy	229.6 ± 50.5	3.21 ± 0.74
<i>P</i>	0.0019	0.0021

Table 4

Effects of isocaloric diets, with different Ca source, on the fat mass of obese women

DIET	BF _{final} (%)	Fat loss (Kg)	Fat loss (% respect the initial one)
Dairy	38.86 ± 0.88	-4.35 ± 0.61	-10.79 ± 1.25
No dairy	43.07 ± 1.12	-2.48 ± 0.86	-6.00 ± 1.77
<i>P</i>	0.0080	0.0911	0.0379

BF_{final} = final body fat content.

Table 5

Effect of low calories diets, containing or not dairy products, on waist circumferences and visceral-abdominal fat thickness in obese pre-menopausal women

DIET	Reduction of waist circumference		Fat thickness (%)	
	cm	% of initial value	SC	VAT
Dairy	4.72 ± 2.52	4.54 ± 2.40	-24.3 ± 15.5	-37.3 ± 20.8
No dairy	4.76 ± 4.35	4.24 ± 3.73	-30.7 ± 10.1	-31.3 ± 15.3
<i>P</i>	NS	NS	NS	NS

SC = subcutaneous; VAT = visceral fat.

3.2. Abdominal-visceral fat

Although doth dietary treatments reduced the body fat, the consumption of low fat dairy foods did not modify the rate of fat loss (Table 5). Neither echographic measurements nor waist circumferences resulted affect by Ca source.

3.3. Ca effects on haematological parameters

Table 6 shows the insulin, reactive C-peptide, leptin, adiponectin and free-fatty acids levels in the blood of the patients fed or not dairy products diets, before and after the energy restriction period. The results did not evidence any significant effect of the food treatments on the main haematological parameters related to the adipose tissue metabolism. The initial values showed that the two groups were homogenous for all the observed parameters, the insulin levels were into the limits both at the beginning and at the end of the experimentation and did not undergo significant changes. Consistently with the observed fat mass decrease, the leptin and adiponectin concentration in blood decreased between the begin and the conclusion of the trial, with any significant variations between the two groups. Instead the IL-6 showed a higher reduction in subjects receiving the “milk diet” compared to diet without dairy products (Table 7). The IL-6 levels showed a significant decrease between the beginning and the end of the experiment, apart from the diet (1.20 vs 0.66 pg/ml; $P < 0.05$).

Both the adiponectin and the unesterified fatty-acid concentration were not influenced by the diet but, while adiponectin decreased between the beginning and the end of the study, NEFA did not show significant time variations.

Table 6
Effect of high/low dairy product diets on haematic parameters, related to the adipose tissue metabolism

DIET	Insulin ($\mu\text{U/mL}$)	C-peptide ($\mu\text{g/L}$)	Leptin (ng/L)	Adiponectin ($\mu\text{g/L}$)	FFA (mM/L)
Initial values*					
Dairy	11.79 ± 2.6	8.05 ± 1.98	40.50 ± 18.2	5.94 ± 3.43	0.45 ± 0.20
No dairy	10.89 ± 3.0	7.33 ± 2.14	43.61 ± 23.8	4.60 ± 2.03	0.43 ± 0.17
<i>P</i>	0.8235	0.3713	0.6236	0.1871	0.7881
Final values					
Dairy	9.72 ± 1.6	9.91 ± 2.1	30.66 ± 20.3	8.41 ± 2.87	0.47 ± 0.08
No dairy	12.16 ± 2.2	12.15 ± 3.3	28.99 ± 20.9	6.80 ± 3.04	0.45 ± 0.13
<i>P</i>	0.3932	0.5846	0.8440	0.2060	0.6222
Variations					
Dairy	-2.19 ± 2.5	1.86 ± 1.8	-14.94 ± 10.1	0.65 ± 1.18	0.01 ± 0.24
No dairy	3.37 ± 3.2	3.68 ± 2.7	-10.22 ± 9.5	1.73 ± 1.46	0.01 ± 0.23
<i>P</i>	0.2038	0.2774	0.2374	0.0869	0.9944
<i>P</i> time effect	0.7782	0.1057	0.0208	0.0050	0.7467

*comprehensive also not concluding subjects; FFA = free fatty acids.

Table 7
Blood levels (mean \pm SE) of IL-6 in premenopausal obese women fed a ipo-caloric diet with different amount of dairy products

	Dairy (pg/ml)	No dairy (pg/ml)	<i>P</i>
Initial value	1.32 ± 0.605	0.98 ± 0.571	0.1559
Final value	0.66 ± 0.490	0.66 ± 0.375	0.9804
Δ IL-6	0.667 ± 0.318	0.320 ± 0.408	0.0191

Glucose metabolism was not affected by dietary treatments, at the end of the experiment blood glucose was not different between the two diets ($89.0 \text{ mg}/100 \text{ ml}$ and $92.6 \text{ mg}/100 \text{ ml}$ for dairy and non dairy diet respectively). HOMA index was 2.18 in diet containing dairy products and 2.26 for dietary regimen lacking of dairy foods and did not result affected by diets.

No significant differences were observed for C-peptide levels between dietary treatments or the beginning and the end of the research.

3.4. Relationship between IL-6 and adipose tissue

IL-6 showed a positive correlation with fat mass and leptin (Table 8), while adiponectin did not show any correlation with antropometric or blood parameters. As expected a significant correlation between leptin and fat mass was detected (Table 8).

The decrease in IL-6 blood level between the start and the end of the experiment and the linear correlation between IL-6 and body mass fat confirm the already demonstrated relationship between fat accumulation and inflammatory status, while the positive link observed between leptin and IL-6 could be due to two different reasons:

- the synthesis of leptin from adipose tissue, as for IL-6;
- the pro-inflammatory role of leptin that could act synergically with IL-6 [17].

Table 8
Correlation between body mass fat and adipokines measured at the end of the experiment

	Fat mass (kg)	Leptin	IL-6	Adiponectin
Fat mass (kg)	.			
Leptin	0.581*	.		
IL-6	0.700**	0.798**	.	
Adiponectin	0.449	0.198	0.299	.

* $P < 0.05$; ** $P < 0.01$.

4. Discussion

4.1. Calcium effects on weight loss and body fat

These results confirmed the hypothesis of the major effectiveness in the lipolytic action of calcium from dairy products, and they are consistent with the results of Summerbell et al. [18], Rosell et al. [19], Pereira et al. [20] and Lee et al. [21] who reported higher weight loss in the subjects fed high dairy Ca diets [18, 19] or diets rich in dairy products, apart from Ca content [20]. But, while in the mentioned studies all people had a BMI lower than 30, in the present study all women were characterised by a first level obesity.

The observed weight loss (7%) is slightly lower than that showed by Zemel [22] (above 10%), but it is comparable the weight loss difference between the two groups: 3,8% showed in the present study and 4,5%, showed by Zemel [22]. This could be due to the different length of the restriction period (168 vs 96 days) and to the different Ca intake in the two experimentation. Indeed, because of ethic reasons, we did not administered low calcium diets to the patients but followed the recommendation fixed by the Italian Society of Human Nutrition (SINU) of 800 mg Ca per day. Recently SINU increased the requirement for Ca to 1000 mg/day. Zemel [22] compared diets from 500 to 1200 mg/die of calcium. Another methodology difference from Zemel's work was the formulation in the present trial of diets containing calcium from different sources, but in equal dosage. So we can effectively attribute the diverse effects of the two diets to the presence of other milk bioactive compounds, which can act in synergy, accelerating the weight loss speed.

Beside, as reported in some works [7, 9, 19, 22–24] and as observed in this study, dairy products rich diets promote an higher body fat mass loss respect to diets lacking in milk products (Table 4). Considering the same protein, energy ad calcium content of the two administered diets, the observed differences can be ascribed to the dairy products consumption; it could be hypothesized diverse involved mechanisms: the higher bioavailability of diary calcium respect of vegetable Ca [25], the fecal fat loss via binding to Ca [26–28], the satiety hormone regulation exercised by milk proteins, such as whey proteins [24, 29, 30] and the synergic action of other milk molecules [31] such as CLA, BCAA, ACE inhibitor and other bioactive peptides, micronutrients such as vitamin D or phosphorus and lactose, that have been shown to reduce fat mass in rats [32]. Recent papers have highlighted the role of vitamin D in the regulation of body weight. Soares et al. [6] reviewed works dealing with the role of vitamin D in the treatment of obesity and reported consistent evidence that this molecule promotes body fat oxidation. However, in our experiment the contribution of low fat dairy products to vitamin D intake is low; the estimated intake of calcitriol was about 5.3 IU/day, which is lower than the supplementation that significantly promoted fat oxidation and ranged from 200 IU/day [33] to more than 5000 IU/day [6]. The conjugated linoleic acid, other than *in vitro* anti-cancer activity, showed also the capacity to increase the lean mass in animal models and humans [34, 35]. The branched-chain amino acids (BCAA), in particular leucine, isoleucine and valine which are abundant in whey, are thought to play an important role in the maintenance of lean body mass [36] and in the reduction of diet-induced obesity [37]. Also angiotensin converting enzyme (ACE) inhibitor may be relevant to adipocyte lipid metabolism: angiotensin II up-regulates adipocyte fatty acid synthase expression, so an ACE inhibitor can mildly attenuate obesity in both obesity and hypertensive patients [22]. These are only some proposed molecules, which may exert a synergic role. Eller and Reimer [38], investigating the role of different protein sources (skim milk powder, casein, whey and soy protein isolate) on weight loss, insulin sensitivity and glucose metabolism regulation, found contradictory results: indeed,

they showed that neither casein nor whey proteins had the same activity of skim milk powder: their data suggest that further studies are needed that examine bioactive molecules of dairy foods.

4.2. Ca and abdominal fat loss

The absence of effect of Ca supplementation on abdominal fat loss does not agree with the results obtained by Zemel [22] or Rosembaum et al. [39]. Both these Authors reported significant and positive effects of Ca supplementation on the loss of abdominal-visceral fat. A possible explanation of the different results compared to our experiment, is the different amount of Ca in the diet. While Ca intake in our subjects was equal to LARN, Rosembaum et al. [39] gave 1050 mg of Ca supplementation, while Zemel [22] compared control diet with insufficient Ca level (500 mg/day) against diet containing 1200 mg of Ca mainly from dairy sources. Rosembaum et al. [39] used drug supplements, while Zemel et al. [22] increased Ca intake using dairy products. In the experiment of Rosembaum et al. [39] the reduction of VAT was not linked to a similar decrease in body weight, while our results were opposite.

4.3. Ca effects on haematological parameters

The adipocytes produce various important circulating factors, such as TNF- α , IL-6, angiotensin, PAI-1, leptin, adiponectin and resistin, involved in different functions as the maintaining of the energetic balance and the promotion or reduction of an inflammation status. In the present work, we also considered some of this factors: IL-6, which has a pro-inflammatory activity [40], adiponectin, which shows an anti-inflammatory function and its secretion decreases in obesity [41, 42] and leptin, that is a protein mainly produced by adipocytes, able to regulate appetite and energy expenditure. In addition leptin exerts an important role as a modulator of inflammatory response [42].

The absence of modifications in these haematic parameters between the two groups may be due to the consumption of diets fulfilling Ca requirements without excess of mineral. While Arnberg et al. [43] and Tandeter et al. [44] reported that milk protein increased C-peptide levels in overweight subjects, we were not able to observe a similar result. A possible explanation for this result could be the inverse relationship between Ca intake from dairy foods and plasma C-peptide found by Wu et al. [45]. We have also to consider that, although the insulin/C-peptide molar ratio is within physiological range, the C-peptide levels detected in our volunteers were sharply higher than ones reported by Abdullah et al. [46] and Ateia et al. [47]. A possible explanation of these high value of C-peptide, could be the finding of Service et al. [48], that C-peptide tend to increase with BMI and in our experiment all the subjects were obese and therefore had high BMI. Adiponectin, leptin and IL-6 are cytokines produced by the adipose tissue, but in contrast to leptin and IL-6, adiponectin secretion is often diminished in obesity [49]. High adiponectin concentration was a protective factor against inflammation and type 2 diabetes. TNF- α was reported as a strong inhibitor of adiponectin activity, so the negative correlation between adiponectin levels and obesity, might be explained by the increased secretion of this cytokine [44]. Our result showed a significant increase in the adiponectin levels after fat loss, in particular in the subjects fed no dairy diet, this result is consistent with the decrease of IL-6, which has a pro-inflammatory activity.

HOMA index was not affected by consumption of dairy foods and value of this index were under the cut-off value (2.6) proposed by Ascaso et al. [16] to define insulin resistance (IR). As a matter of fact, our volunteers were not affected by diabetes neither impaired glucose tolerance, so the absence of IR is not a surprise. The initial normal glucose homeostasis can explain the absence of dietary effect on blood glucose and HOMA index.

5. Conclusions

Beyond to confirm the positive effects on body weight loss and lipolysis of dietary Ca also in a dosage equal to the recommended one, this work unequivocally showed that diets containing Ca from dairy products increased weight loss compared to iso-energetic and iso-not dairy-Ca ones. This enhanced activity may be attributed to a number of bioactive molecules present in milk, which can act in synergy with calcium. Nevertheless, the intake of one of these molecules, vitamin D, in this experiment was estimated to be lower than the active dose and consequently the enhanced activity of dairy Ca should not be related to calcitriol intake.

Adipokines were not affected by the different calcium sources, however weight loss lead to an increase in adiponectin synthesis and a decrease of IL-6 levels. By and large, these results confirm the importance of fat loss for decreasing organism inflammation in obese subjects.

Acknowledgments

This research was supported by a grant from “Fondazione Romeo ed Enrica Invernizzi”, Milano, Italy.

References

- [1] OECD, Organisation for Economic Co-operation and Development. Obesity update 2012; 2012, [cited 20 Dec 2012]. Available from www.oecd.org/dataoecd/1/61/49716427.pdf.
- [2] Berghöfer A, Pischon T, Reinhold T, Apovian CM, Sharma AM, Willich SN. Obesity prevalence from a European perspective: A systematic review. *BMC Public Health* 2008;8:200-9.
- [3] McCarron DA, Morris CD, Henry HJ, Stanton JL. Blood pressure and nutrient intake in the United States. *Science*. 1984;224(4656):1392-8.
- [4] Trevisan M, Krogh V, Farinano E, Panico S, Mancini M. Calcium rich-foods and blood pressure: Findings from the Italian National Research Council (The Nine Communities Study). *Am J Epidemiol*. 1988;127(6):1155-63.
- [5] Zemel MB, Shi H, Greer B, Di Rienzo D, Zemel PC. Regulation of adiposity by dietary calcium. *FASEB J*. 2000;14(9):1132-8.
- [6] Soares MJ, Murhadi LL, Kurpad AV, Cang She Ping-Delfos WL, Piers LS. Mechanistic roles for calcium and vitamin D in the regulation of body weight. *Obesity Review*. 2012;13(7):592-605.
- [7] Zemel MB. The role of dairy foods in weight management. *J Am Coll Nutr*. 2005;24(6 Suppl):537S-46S.
- [8] Weaver CM, Campbell WW, Teegarden D, Craig BA, Martin BR, Singh R, Braun MM et al. Calcium, dairy products and energy balance in overweight adolescents: A controlled trial. *Am J Clin Nutr*. 2011;94(5):1163-70.
- [9] Gunther CW, Legowski PA, Lyle RM, McCabe GP, Eagan MS, Peacock M, Teegarden D. Fat oxidation and its relationship to serum parathyroid hormone in young women enrolled in a 1 year dairy calcium intervention. *Am J Clin Nutr*. 2005;82(4):1228-34.
- [10] Lohman TG, Roche AF, Martorel R. Anthropometric Standardization Reference Manual. Champagne: Human Kinetics Books; 1988.
- [11] Segal KR, Burastero S, Chun A, Coronel P, Pierson Jr RN, Wang J. Estimation of extracellular and total body water by multiple-frequency bioelectrical-impedance measurement. *Am J Clin Nutr*. 1991;54(1):26-9.
- [12] Deurenberg P, Van der Kooy K, Leenen R, Weststrate JA, Seidell JC Sex and age specific prediction formulas for estimating body composition from bioelectrical impedance: A cross-validation study. *Int J Obes*. 1991;15(1):17-25.
- [13] Armellini F, Zamboni M, Rigo L, Todesco T, Bergamo-Andreis IA, Procacci C, Bosello O. The contribution of sonography to the measurement of intra-abdominal fat. *J Clin Ultrasound*. 1990;18(7):563-7.
- [14] Isbell TR, Klesges RC, Meyers AW, Klesges LM. Measurement reliability and reactivity using repeated measurements of resting energy expenditure with a face mask, mouthpiece, and ventilated canopy. *J Parenter Enteral Nutr*. 1991;15(2):165-8.
- [15] Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *Nutrition*. 1990;6(3):213-21.
- [16] Ascaso JF, Pardo S, Real JT, Lorente RI, Priego A, Carmena R. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. *Diabetes Care*. 2003;26(12):3320-5.
- [17] Fantuzzi G. Three questions about leptin and immunity. *Brain Behav Immun*. 2009;23(4):405-10.
- [18] Summerbell CD, Watts C, Higgins JPT, Garrow JS. Randomised controlled trials of novel simple and well supervised weight reducing diets in outpatients. *Br Med J*. 1998;317(7171):1487-9.
- [19] Rosell M, Hakansson NN, Wolk A. Association between dairy food consumption and weight change over 9 years in 19352 perimenopausal women. *Am J Clin Nutr*. 2006;84(6):1481-8.
- [20] Pereira MA, Jacobs DR, Van Horn L, Slattery ML, Kartashov AI, Ludwig DS. Dairy consumption, obesity and the insulin resistance syndrome in young adults. *JAMA*. 2002;287(16):2081-89.
- [21] Lee H, Cho J, Lee HH, Kim C, Cho E. Intakes of Dairy Products and Calcium and Obesity in Korean Adults: Korean National Health and Nutrition Examination Surveys (KNHANES) 2007-2009. *PlosOne*. 2014;9(6):e99085.
- [22] Zemel MB. Role of calcium and dairy products in energy partitioning and weight management. *Am J Clin Nutr*. 2004;79(5):907-12.
- [23] Barr SI. Increased dairy product or calcium intake: Is body weight or composition affected in humans ? *J Nutr*. 2003;133(1):245S-8S.
- [24] Major GC, Alarie FP, Doré J, Tremblay A. Calcium plus vitamin D supplementation and fat mass loss in female very low-calcium consumers: Potential link with a calcium-specific appetite control. *Br J Nutr*. 2009;101(5):659-63.
- [25] Park YW, *Bioactive Components in Milk and Dairy Products*, 2009, Wiley-Blackwell editor.
- [26] Magnano San Lio E, Bruschi S, Mulazzi A Calabrese G, Piva G, Rossi F. *In vivo* and *in vitro* digestibility of the calcium contained in foods of animal and plant origin. *Med J Nutr Metabol*. 2011;4(2):105-10.

- [27] Jacobsen R, Lorenzen JK, Toubro S, Krog-Mikkelsen I, Astrup A. Effect of short-term high dietary calcium intake on 24-hours energy expenditure, fat oxidation, and fecal fat excretion. *Int J Obes.* 2005;29(3):292-301.
- [28] Lorenzen JK, Nielsen S, Holst JJ, Tetens I, Rehfeld JF, Astrup A. Effect of dairy calcium or supplementary calcium intake on postprandial fat metabolism, appetite, and subsequent energy intake. *Am J Clin Nutr.* 2007;85(3):678-87.
- [29] Veldhorst MA, Nieuwenhuizen AG, Hochstenbach-Waelen A, van Vught AJ, Westerterp KR, Engelen MP, Brummer RJ, Deutz NE, Westerterp-Plantenga MS. Dose-dependent satiating effect of whey relative to casein or soy. *Physiol Behav.* 2009;96(4-5):675-82.
- [30] Bendtsen LQ, Lorenzen JK, Bendtsen NT, Rasmussen C, Astrup A. Effect of Dairy Proteins on Appetite, Energy Expenditure, Body Weight, and Composition: A Review of the Evidence from Controlled Clinical Trials. *Adv Nutr.* 2013;4(4):418-38.
- [31] Huth PJ, DiRienzo DB, Miller GD (2006) Major scientific advances with dairy foods in nutrition and health. *J Dairy Sci.* 2006;89(4):1207-21.
- [32] Goseki-Sone M, Maruyama R, Sogabe N, Hosoi T. Effect of dietary lactose on long-term high-fat diet-induced obesity in rats. *Obesity.* 2007;15(11):2605-13.
- [33] Major GC, Chaput JP, Ledoux M, St-Pierre S, Anderson GH, Zemel MB, Tremblay A. Recent developments in calcium-related obesity research. *Obesity Rev.* 2008;9(5):428-45.
- [34] Larsen TM, Toubro S, Astrup A. Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: Evidence from animal and human studies. *J Lipid Res.* 2003;44(12):2234-41.
- [35] Terpstra AHM (2004) Effect of conjugated linoleic acid on body composition and plasma lipids in humans: An overview of the literature. *Am J Clin Nutr.* 2004;79(3):352-61.
- [36] Layman DK, Walker DA. Potential importance of leucine in treatment of obesity and the metabolic syndrome. *J Nutr.* 2006; 136(Suppl 1):319S-23S.
- [37] Zhang Y, Guo K, LeBlank RE, Loh D, Schwartz GJ, Yu YH. Increasing dietary leucine intake reduces diet-induced obesity and improves glucose and cholesterol metabolism in mice via multi-mechanisms. *Diabetes.* 2007;56(6):1647-54.
- [38] Eller L, Reimer RRA. A high calcium, skim milk powder diet results in a lower fat mass in male, energy-restricted, obese rats more than a low calcium, casein or soy protein diet. *J Nutr.* 2010;140(7):1234-41.
- [39] Rosenblum JL, Castro VM, Moore CE, Kaplan LM. Calcium and vitamin D supplementation is associated with decreased abdominal visceral adipose tissue in overweight and obese adults. *Am J Clin Nutr.* 2012;95(1):101-8.
- [40] Gustafson B. Adipose tissue, inflammation and atherosclerosis. *J Atheroscler Thromb.* 2010;17(4):332-41.
- [41] Fantuzzi G. Adipose tissue, adipokines and inflammation. *J Allergy Clin Immunol.* 2005;115(5):911-19.
- [42] Matsuzawa Y. Establishment of a concept of visceral fat syndrome and discovery of adiponectin. Review. *Proc Jpn Acad Ser B Phys Biol Sci.* 2010;86(2):131-41.
- [43] Arnberg K, Mølgaard C, Michaelsen KF, Jensen SM, Trolle E, Larnkjær A. Skim milk, whey and casein increase body weight and whey and casein increase the plasma C-peptide concentration in overweight adolescents. *J Nutr.* 2012;142(12):2083-90.
- [44] Tandeter H. Improvement of glycaemic control and elevation of C-peptide following a diet free of dairy products in an insulin-treated patient with type 2 diabetes with ulcerative colitis. *Br Med J Case Rep.* 2009. doi: 10.1136/bcr.07.2008.0389.
- [45] Wu T, Willett WC, Giovannucci E. Plasma C-peptide is inversely associated with calcium intake in women and with plasma 25-hydroxy vitamin D in men. *J Nutr.* 2009;139(3):547-54.
- [46] Abdullah A, Hasan H, Raigangar V, W. Bani-Issa W. C-peptide versus insulin: Relationships with risk biomarkers of cardiovascular disease in metabolic syndrome in young arab females. *Int J Endocrinol.* 2012. doi: 10.1155/2012/420792
- [47] Ateia S, Rusu E, Cristescu V, Enache G, Cheta DM, Radulian G. Proinsulin and age in general population. *J Medicine Life.* 2013;6(4):424-9.
- [48] Service FJ, O'Brien PC, Kao PC, Young WF Jr. C-peptide suppression test: Effect of gender, age and body mass index; implications for the diagnosis of insulinoma. *J Clin Endocrinol Metab.* 1992;74 (1):204-10.
- [49] Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol.* 2010;316(2):129-39.