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ORIGINAL CONTRIBUTION

Effect of dietary calcium (Ca) on body composition and Ca metabolism during growth in genetically obese (β) male rats

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Abstract
Introduction Obese β rats may be a suitable model to evaluate the association between calcium intake (CaI) and obesity during growth.
Objective The present study comparatively evaluated Ca absorption and retention, and changes in body composition in spontaneously genetically obese (β) male rats fed three different dietary Ca levels: high 0.9% (HCa); normal: 0.5% (NCa); low: 0.2% (LCa).
Methods Pregnant rats were fed isocaloric diets which varied in Ca content only. Male pups continued feeding the same maternal diet until postnatal day 60. The percentage of Apparent Ca absorption (CaA %), Ca balance (CaB), body composition, glucose, triglycerides (TGL), and insulin levels were evaluated.
Results Food consumption and body weight (BW) were higher in Group LCa than in Groups NCa and HCa ($p < 0.01$); no differences were observed between the latter two groups. Group LCa presented the highest body fat, liver weight, perigonadal and retroperitoneal fat ($p < 0.05$); conversely, body ashes and total skeleton bone mineral content were significantly lower compared with animals in both the NCa ($p < 0.01$) and HCa groups ($p < 0.01$). CaB (mg/day) reached a plateau at the highest CaI (mg/day) value ($r = 0.985$, $p < 0.001$). CaA%, serum glucose, insulin, and TGL levels rose as CaI decreased ($p < 0.01$).
Conclusions Although further studies are required, low Ca consumption in this strain of rats could modulate BW inducing changes in several lipid metabolism parameters, which in turn lead to an increase in body fat.

Keywords Low calcium diet · Bone mass · Fat

Abbreviations

Ca	Calcium
CaI	Calcium intake
HCa, NCa, LCa	High, normal, and low calcium diets, respectively
CaA%	Percentage of apparent calcium absorption
CaB	Calcium balance
TGL	Triglycerides
BW	Body weight
Ca^{++}	Ionic Ca
NHANES III	National Health and Nutrition Examination Survey III
ENNyS	National Nutrition and Health Survey
CARDIA	Coronary Artery Risk Development in Young Adults

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AIN′93	American Institute of Nutrition Rodent Diets Recommendations settled in 1993
F	Feces
U	Urine
Ca F	Fecal Ca
CaU	Urinary Ca
BMC	Bone mineral content
DXA	Dual energy X-ray absorptiometry
CV	Coefficient of variation
AOAC	Association of Official Analytical Chemists Methods
N	Nitrogen
P	Phosphorus
SE	Standard error
ANOVA	Analysis of variance
mg/L	Milligram per liter
FAS	Fatty acid synthetase
$[Ca^{++}]_i$	Ionic Ca intracellular concentration

Introduction

Calcium (Ca) is essential to achieve optimum peak bone mass during growth and development, and maintain skeletal integrity in adult life. More than 99% of total body Ca is in the skeleton, where it is associated with phosphate to form apatite crystals. The remaining Ca is present in soft tissues, where ionic Ca (Ca^{++}) is involved in a great number of physiological functions, such as regulation of nerve excitability, muscle contraction and blood coagulation, among others.

In 2004, Zemel et al. [1] suggested that Ca is also involved in the modulation of energy metabolism, exerting an "anti-obesity" effect; the authors confirmed their finding in later studies conducted in humans and in mice [2–4]. This theory would come to link two increasing worldwide problems: the well-known high prevalence of low Ca intake (CaI) and the increase in the prevalence of human overweight and/or obesity. Several studies have shown that adiposity and CaI are inversely correlated, especially during growth [5–8]. In addition, epidemiological observations from the National Health and Nutrition Examination Survey III (NHANES III) [2], the Coronary Artery Risk Development in Young Adults (CARDIA) study [5, 7], and the post-analysis of the data from the National Nutrition and Health Survey (ENNyS) conducted in Argentina during 2004–2006 [10] showed an inverse relation between both overweight and obesity, and CaI.

The effect of different dietary Ca contents on bone metabolism has been studied extensively in normal rats. However, to our knowledge, this effect has not been evaluated in an inbred rat strain that spontaneously develops obesity during the prepuberal period, as is the IIMBβ strain [9]. Moreover, this strain of rats may be considered a suitable model to evaluate the relation between CaI and body composition, which remains a controversial subject to date. Based on the above, the aim of the present study was to evaluate Ca absorption and retention, and changes in body composition in spontaneously obese rats feeding different dietary Ca content: low, normal, and high.

Materials and methods

Animals

Virgin female IIMb rats (250–300 g) were obtained from the Lipid and Lipoprotein Laboratory of the department of Clinical Biochemistry, Rosario National University. They were housed at controlled room temperature (21 ± 1 °C) and humidity of 55 ± 10%, under 12-h light/dark cycles.

IIMb/β rats develop obesity and type II diabetes from puberty onwards. They were obtained by genetically environmental maladjustment and a high degree of inbreeding. These obese rats also develop hypertryacilglyceridemia without hypercholesterolemia, and their glucose intolerance progresses to type II diabetes and obesity-related hypertension [11].

Throughout the experimental period, rats were allowed to access deionized water and food ad libitum. They were maintained in keeping with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the protocol was approved by the Bioethics Committees of the Universities of Buenos Aires and Rosario.

Experimental design

Adult virgin female rats were mated by placing one male rat in a cage with four females. From pregnancy to the end of lactation, the dams were housed in individual stainless steel cages and were randomly assigned to receive one of three studied isocaloric diets, which only varied in Ca content: Low (LCa), Normal (NCa), and High (HCa) (Table 1) [12].

Maternal body weights were recorded once a week. Litter body weights and number of females and males were recorded at delivery and within 24 h. The number of pups was adjusted to 8–9 per dam. In order to maintain such number of pups, female offsprings were kept during lactation. From birth to weaning, pup body weight (BW) was registered twice a week. At weaning, male pups ($n = 8$ per diet) continued feeding the maternal diet until postnatal day 60, while female pups were euthanized. Food consumption was recorded 3 times a week, BW was recorded once a week, and food efficiency (g/g) was calculated according to the following equation:

Table 1 Composition of the three experimental diets

Diet	LCa	NCa	HCa
Energy (Kcal)	395	395	395
Energy (KJ)	1,675	1,675	1,675
Protein (g)*	17.0**	17.0**	17.0**
Lipids (g)**	7.0	7.0	7.0
Calcium-free salts mixture	4.0#	4.0#	4.0#
Water-soluble vitamins***	0.25#	0.25#	0.25#
Fat-soluble vitamins***	0.50#	0.50#	0.50#
Choline	0.15	0.15	0.15
Cellulose	5.0	5.0	5.0
Dextrin&	to complete 100 g		
Calcium (g)	0.2****	0.6****	0.9****
Phosphorus (g)	0.6	0.6	0.6

* Potassium Caseinate, Nestlé Argentina S.A., containing 85.1% of protein and 0.095 g% of Ca

** Commercial soy oil

*** Vitamins (individual components from Sigma, Missouri, USA), 5% cellulose and 0.71% choline citrate (food grade, Anedra, Argentina)

**** $CaCO_3$ (food grade individual components, Anedra, Argentina), was added to obtain the required Ca concentration

Composition according to AIN′93

& Dextrin was added as carbohydrate source to reach 100 g of diet

$$\text{Food efficiency} = \text{Food intake(g)}/\text{increase in BW(g)}$$

Fasting blood samples were collected under anesthesia (0.1 mg ketamine hydrochloride/100 g BW and 0.1 mg acetopromazine maleate/100 g BW) at the end of the experiment ($T = 60$). The animals were then killed by CO_2, and intraperitoneal and retroperitoneal fat and liver were removed and weighed.

Diets

The 3 experimental diets were prepared according to the American Institute of Nutrition Rodent Diets Recommendations settled in 1993 (AIN′93) [12]. Ca content was provided by CO_3Ca (Analytical grade, Anedra, Argentina) to supply the 3 different Ca contents: HCa: 0.9% Ca; NCa: 0.5% Ca; LCa: 0.2% Ca. The composition of each diet is outlined in Table 1.

Balance and apparent Ca absorption

During the last 3 days of the experience, the animals were housed individually in plastic metabolic cages, and food consumption, feces (F), and urine (U) samples were collected to calculate Ca absorption (CaA) and Ca balance (CaB) [13]. Apparent CaA, expressed as a percentage of CaI (CaA %), and CaB (mg) were calculated according to the following equations:

$$\text{CaA } \% = (\text{CaI} - \text{CaF}/\text{CaI}) \times 100$$

$$\text{CaB} = (\text{CaI} - \text{CaF} - \text{CaU}).$$

Densitometry

At the end of the experience and before killing, total skeleton bone mineral content (BMC) was determined "in vivo" under light anesthesia, by dual energy X-ray absorptiometry (DXA) with a whole body scanner and a specifically designed software for small animals (DPX Alpha, Small Animal Software, Lunar Radiation Corp. Madison WI), as described in a previous report [14]. In brief, all rats were scanned using an identical scan procedure. Precision was assessed by measuring one rat five times with repositioning between scans, on the same day and on different days. The coefficient of variation of BMC (CV) was 3.0%.

Analytical procedures

Body composition was determined according to the Association of Official Analytical Chemists (AOAC) methods [15]. Water was measured by desiccation to constant weight, in an oven at 100 °C. Total body fat content was determined in the ground, dried carcasses by extraction with petroleum ether in a Soxhlet apparatus. White and crystalline ashes were obtained at 550 °C. Nitrogen (N) was determined by Kjeldahl's method, and the percentage of protein content was calculated as N × 6.25 (% protein = % N × 6.25) [16]. Feces were dried in an oven at 42 °C during 12 h, ground to 0.5–1.0 mm mesh, and dried again at 105 °C for 2 h. Dried fecal samples (1.5–2.0 g) were homogenized in a Potter-Elvejahn device, and lipids were extracted with chloroform/methanol (2:1 v/v). Total lipids were determined gravimetrically after solvent evaporation. Defatted feces were dried under an IR lamp and powdered in a processing machine equipped with a titanium blade. Feces and diets were digested with nitric acid in a microwave oven using Parr bombs to determine Ca and phosphorus (P) content [17]. Ca concentration in each diet, feces, urine, and ashes was determined by atomic absorption spectrophotometry [18]. Lanthanum chloride (6,500 mg/L in the final solution) was added to avoid interferences. P content in ashes was determined according to Gomori's method [19].

At the end of the experience ($T = 60$), serum glucose, cholesterol, and triglycerides (TGL) were determined using conventional enzymatic methods, and insulin was determined by enzyme immunoassay (Rat/Mouse Insulin ELISA Kit, Millipore, Billerica, MA, USA).

Statistical methods

Results were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of

variance (ANOVA), and the Bonferroni multiple comparisons test was performed when significant differences were observed. Statistical analyses were carried out using SPSS for Windows 11.0 (SPSS, Inc. Chicago, IL). A value of P below 0.05 ($p < 0.05$) was considered significant.

Results

Pregnancy rate of dams feeding the LCa diet was low; two dams delivered less than 8 pups and were eliminated from the study. The mean offspring number and sex ratio (male: female) of groups LCa, NCa, and HCa were: 9 ± 2 and 9 ± 4; 10 ± 2 and 4:5; and 4:5 and 5:5, respectively.

Impact on body weight and composition

No significant differences in pup BW were observed among groups at delivery (LCa: 5.9 ± 0.6 g; NCa: 5.4 ± 0.3 g; and HCa: 5.1 ± 0.7 g); nevertheless, BW of pups fed the LCa diet was slightly higher.

Throughout the entire experience, food consumption was significantly higher in the LCa group compared with the other two studied groups ($p < 0.01$); no significant differences were observed between the two latter groups (Table 2).

Figure 1 shows the changes in BW observed in each of the 3 studied groups throughout the experience. As of weaning, the LCa group showed significantly higher BW values than the NCa and HCa groups ($p < 0.01$), which did not differ.

Despite the higher food intake in the LCa group, no differences in food efficiency, calculated as food intake/BW (g/g) ratio, were observed among the three studied groups throughout the study (Table 2) or during the balance period (Table 4).

Body composition, expressed as a percentage of BW, and total content are shown in Table 2. No significant differences in water content to BW ratio were observed among groups (Table 2). The LCa group showed the highest significant content of total fat and fat normalized by BW compared with both the NCa and HCa groups ($p < 0.01$). As a result of the high BW value, the LCa group showed the highest total protein content ($p < 0.01$) in spite of exhibiting the lowest protein to BW ratio, which only reached statistical significance when compared with the NCa group ($p < 0.01$). Total ash content was significantly lower in the LCa and HCa groups as compared to the NCa group ($p < 0.01$); conversely, ash content to BW ratio correlated with dietary Ca content, with the lowest significant value corresponding to the LCa group ($p < 0.0001$) and no significant differences between the NCa and HCa groups.

A significant linear correlation ($r = 0.78$; $p < 0.012$) was found when the individual points of total ash content were plotted against lean mass, expressed according to the following equation (Fig. 2):

$$\text{Lean mass} = \text{BW} - \text{total fat content} - \text{total water content} - \text{total ashes}.$$

Impact on glucose and energy metabolism

Table 3 shows fat intake, fecal excretion, and fat absorption percentage corresponding to the last three days of experience. No differences in food consumption were observed among the studied groups (22.9 ± 4.7;

Table 2 Food consumption throughout the experience, final body weight and body composition at the end of the experience. At weaning, IIMb/β rats male pups continued feeding their mother's diets: LCa, NCa, or HCa up to reach 60 days of age

	LCa	NCa	HCa
Food consumption (g/day)	18.9 ± 2.1	15.9 ± 2.9*	16.0 ± 4.0*
Final BW (g)	281 ± 40	230 ± 42*	198 ± 16*
BW increase (g/day)[a]	6.4 ± 1.0	5.3 ± 0.8*	4.7 ± 1.0*
Food efficiency (g/g)[a]	0.34 ± 0.04	0.34 ± 0.05	0.31 ± 0.03
Body composition			
Water (g/100 g BW)	67.3 ± 2.2	66.7 ± 2.8	69.1 ± 3.5
Body fat (g/100 BW)	15.9 ± 1.5	13.1 ± 2.2*	12.6 ± 2.2*
Total body fat (g/rat)	45.1 ± 10.0	38.6 ± 9.7*	32.7 ± 1.8*
Protein (N × 6.25) (g/100 g BW)	14.8 ± 2.6**	17.3 ± 1.7	15.8 ± 2.1**
Total body protein (g/rat)	41.1 ± 4.5	30.3 ± 5.2*	31.2 ± 2.2*
Ashes (g/100 g BW)	2.01 ± 0.21	2.59 ± 0.18*	2.65 ± 0.36*
Total body ashes (g/rat)	5.5 ± 0.9	6.2 ± 1.1*	4.9 ± 0.3*,**

Data are expressed as mean ± SD (*) $p < 0.01$ compared to LCa group; (**) $p < 0.01$ compared to NCa group

[a] Data calculated throughout the experience. Differences were analyzed by Bonferroni test after ANOVA

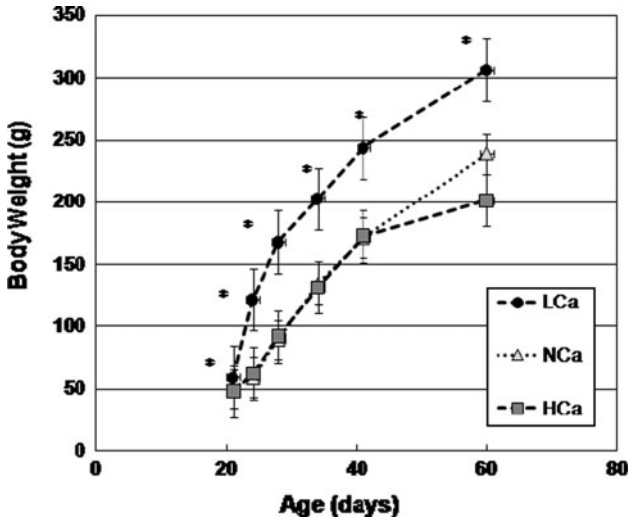

Fig. 1 Changes in body weight throughout the experience. Data are presented as mean ± SD. (*): $p < 0.01$ significantly different from NCa and HCa groups. Differences were analyzed by Bonferroni test after ANOVA

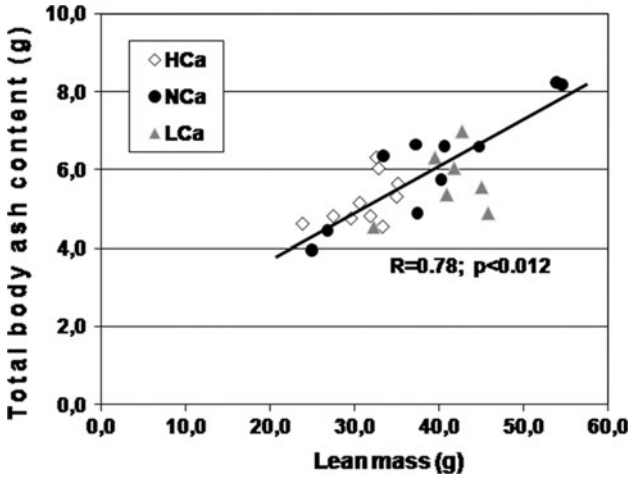

Fig. 2 Correlation between total body ash (g) and body lean mass (g). ($y = 0.106x + 1.766$; $R = 0.78$; $p < 0.012$)

25.2 ± 4.8; and 24.6 ± 1.7 mg/day for groups LCa, NCa, and HCa, respectively). As a result, no differences in fat intake were found among the three studied groups, either. Fecal fat excretion was significantly higher in the HCa group compared with both the LCa and NCa groups ($p < 0.01$), which showed no significant differences. However, fat absorption percentage did not differ among the three studied groups.

Adipose perigondal and retroperitoneal pads, liver weight, and serum levels of glucose, insulin, and TGL rose as CaI decreased ($p < 0.01$), whereas cholesterol levels were significantly higher in the NCa group compared with both the LCa and HCa groups ($p < 0.05$); no differences were observed between the latter groups (Table 3).

Impact on Ca absorption and retention

Because there were no differences in food intake during the period when absorption was evaluated, CaI was directly related to the dietary Ca content and the LCa group had the lowest CaI ($p < 0.01$) (Table 4).

Similarly to ash content, total body Ca and P contents did not differ between the LCa and HCa groups, both of which showed significantly lower values than the NCa group ($p < 0.001$) (Table 4). Body Ca and P contents normalized by BW were significantly lower in the LCa group compared with both the NCa and HCa groups, and no differences were observed between the latter. In spite of these values, no differences in the Ca/P ratio were observed between the LCa and NCa groups and both groups exhibited significantly lower Ca/P ratio values compared with the HCa group (1.36 ± 0.04 and 1.36 ± 0.03 vs. 1.54 ± 0.05, respectively; $p < 0.05$).

Fecal and urinary Ca excretion during the absorption study period correlated significantly with dietary Ca content ($p < 0.001$ and $p < 0.01$, respectively). CaB (mg) showed direct correlation and CaA (% of CaI) showed inverse correlation with the Ca content in the diets (Table 4). Indeed, CaB (mg/day) plotted against CaI (mg/day) showed a positive function which reached a plateau in the high ranges of CaI ($r = 0.985$, $p < 0.001$) (Fig. 3). CaA% was significantly lower in the HCa group as compared to the LCa and NCa groups ($p < 0.01$), and no differences were observed between the latter.

Impact on densitometry

Total skeleton BMC normalized by BW (BMC/100 g BW) was significantly lower in the LCa group as compared to both the NCa ($p < 0.01$) and HCa ($p < 0.01$) groups; in addition, BMC/100g BW was lower in the HCa group than in the NCa group ($p < 0.01$) (Table 4).

Discussion

In addition to the well-established role of Ca in bone and mineral metabolisms, there has been considerable recent interest in the hypothesis that CaI modulates body fat stores. The inbred IIMb/beta line of rats may be an interesting and suitable model to study the interrelationship between obesity and dietary Ca intake during the growth period because they develop progressive obesity and type 2 diabetes from puberty onwards, regardless of energy intake.

Impact on body composition

The results of the present report strongly suggest that, as previously observed in wild-type male mice [20], dietary

Table 3 Laboratory findings, fat absorption measurement, liver weight and fat pad

	LCa	NCa	HCa
Glucose (mg/dl)	207.0 ± 11.0	151.9 ± 21.9*	111.8 ± 25.5*,**
Cholesterol (mg/dl)	64.5 ± 3.1**	78.2 ± 5.4	67.4 ± 8.0**
Triglycerides (mg/dl)	272.6 ± 39.9	205 ± 35.2*	216.4 ± 26.8*
Insulin (mg/dl)	6.87 ± 2.24	4.07 ± 0.93*	1.92 ± 0.75*,**
Fat intake (mg/day)	1,686.0 ± 228.3	1,765 ± 315.9	1,722.2 ± 106.7
Fecal fat (mg/day)	39.3 ± 11.1	42.9 ± 14.1	54.2 ± 13.1*,**
Fat absorption (%)	96.4 ± 0.9	97.5 ± 0.6	97.5 ± 0.5
Liver weight (g)	15.6 ± 0.7	12.3 ± 2.6*	10.2 ± 1.2*
Perig. + Retrop.fat (g)	13.5 ± 0.72	10.2 ± 2.58*	7.43 ± 1.55*,**
% (Perig. + Retrop.) fat/BW	5.34 ± 0.24	4.36 ± 0.48*	3.77 ± 0.46*,**

The serum levels of glucose, insulin, cholesterol, and triglycerides were analyzed at the end of the study. Fat intake, fecal fat, and percentage of fat absorption were assayed during the last three days of experience. Liver weight, perigonadal plus retroperitoneal fat as total and normalized by body weight were obtained at killing

Data are expressed as mean ± SD (*) $p < 0.01$ compared to LCa group; (**) $p < 0.01$ compared to NCa group. Differences were analyzed by Bonferroni test after ANOVA

Table 4 Calcium balance, body calcium and phosphorus content and bone densitometry

	LCa	NCa	HCa
Food efficiency (g/g) during the balance period	0.33 ± 0.04	0.36 ± 0.04	0.36 ± 0.05
Ca intake (mg/day)	37.9 ± 4.2	79.6 ± 14.6*	144.1 ± 36.3*,**
Fecal Ca (mg/day)	3.9 ± 4.0	15.7 ± 5.9*	80.8 ± 16.1*,**
Urinary Ca (mg/day)	0.7 ± 0.2	1.0 ± 0.6*	1.3 ± 0.4*,**
Apparent Ca absorption (%)	92 ± 8†	88 ± 1†	64 ± 6
Body Ca (mg/100 g BW)	567.5 ± 60.4	802.3 ± 100.8*	814.8 ± 114.4*
Total body Ca (mg/rat)	1656 ± 119	1818 ± 298*	1639 ± 266**
Body P (mg/100 g rat)	418.4 ± 22.1	589.9 ± 77.2*	530.7 ± 89.6*
Total body P (mg/rat)	1281 ± 359	1457 ± 309*	1068 ± 274*,**
Calcium balance (mg/day)	44.6 ± 6.8	99.8 ± 6.9*	139.0 ± 11.3*,**
BMD (mg/cm^2)	222.3 ± 9.0	234.6 ± 7.2*	232.6 ± 4.4*
BMC/BW (g/100 g BW)	1.04 ± 0.20	1.44 ± 0.32*	1.27 ± 0.18*,**

Ca intake, fecal and urinary Ca excretions, Ca balance, Ca absorption (%) were determined during the last three days of experience. Total body Ca and P content, body Ca and P normalized by body weight, total skeleton bone mineral density, and bone mineral content normalized by body weight were obtained by DXA at the end of the experience

Data are expressed as mean ± SD (*) $p < 0.01$ compared with LCa group; (**) $p < 0.01$ compared with NCa group; $^{\dagger}p < 0.01$ compared with HCa group. Differences were analyzed by Bonferroni test after ANOVA

Ca may play a role in modulating BW, in spontaneously obese male IIMb/β rats.

According to the results obtained in the present study, rats fed the low isocaloric Ca diet exhibited a significant increase in food intake, BW gain, fat pad mass, and a decrease in protein content per kg of BW. Even though the differences in BW were observed from weaning, they may have been present before, during lactation. Indeed, the dams received the different studied diets from mating, and it is known that pups begin to consume a mixed diet (milk plus solid) approximately during the last week before weaning [21]. In this regard, it is important to take into account that a previous study conducted at our laboratory showed that mineral milk composition was not affected in Wistar dams fed diets containing the Ca levels used in the present report [22]. The results also showed that these different maternal Ca intakes during pregnancy affected neither fetal size nor weight nor body mineral content of the pups [23]. Thus, the same model was applied in the present research in IIMb rats, with the aims to highlight the effect of dietary calcium.

No differences in the percentage of fat absorption were observed during the three days of the balance period; however, a slight decrease in the amount of total fecal fat excretion was observed in the LCa group. According to these results, it could be assumed that although the effect of

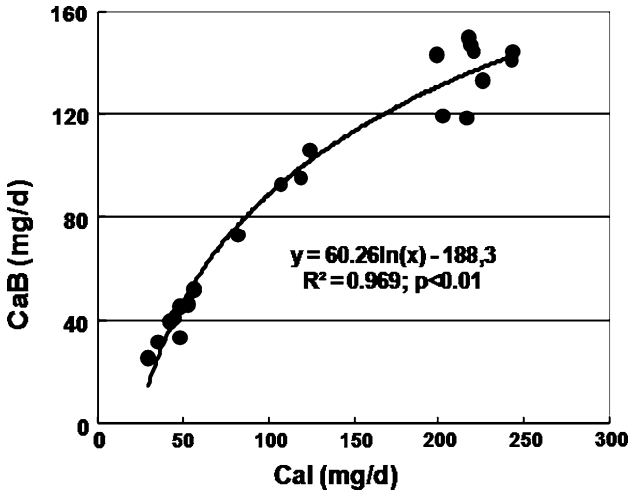

Fig. 3 Ca balance (mg/day) as a function of Ca intake (mg/day) ($y = 60.26 \ln(x) - 188.3$; $R^2 = 0.969$; $p < 0.01$)

low dietary Ca on increasing body fat accumulation may likely be quite small, it is high enough for the increase to reach significant levels throughout the experience. In addition, rats fed the low Ca diet showed an increase in liver weight that actually reflects higher body fat content, evidenced by the increment in adipose perigonadal and retroperitoneal pads as well as by the sum of both expressed as a percentage of BW. This increase could be the result of the higher food consumption in the LCa group; however, it should be kept in mind that there were no differences in food efficiency, calculated as food intake/BW (g/g) ratio, among the three studied groups. It is also important to take into account that even though the process of fat and lean mass accumulation may have similar energy costs, lean mass per kg is metabolically more active and requires greater energy utilization than fat mass [24]. Hence, it is important to take into account that the increase in lean body mass observed in normal and moderate high Ca diets may shift energy away from fat stores.

Daily food intake would decrease if the food were unpalatable [25]. Therefore, no aversion to food was observed in the present report since no differences in food intake were observed between rats fed the isocaloric diets, that is, the diet supplying the recommended AIN 93 Ca levels (0.5%) and that supplying a moderately higher level (0.9%). In spite of this, rats fed the moderate Ca diet tended to show lower fat content and BW gain rate. A similar finding was observed by Zhang Q and Tordoff MG [25] in adult female Sprague–Dawley rats that were allowed to choose between pairs of normal-energy density diets with different Ca levels. The authors observed a higher food intake in rats fed the low Ca diet.

The inverse relationship between CaI and BW gain remains somewhat controversial. Several mechanisms have been proposed as potentially contributing to, and partly explaining, the overall impact of dietary Ca on BW or body fat mass. One suggested hypothesis is that dietary Ca levels may affect appetite and food intake [26]. In this regard, mammals may have evolved to respond to specific dietary components that act as indicators of dietary availability, so that, when food is plentiful, a mineral present in the diet (e.g., Ca) acts as the signal to reduce body fat mass accumulation [26]. Conversely, as seen in the present report, the low level of dietary Ca during a high growth rate period may serve as an indicator to promote food intake, thus protecting the body against such deficiency. Indeed, food intake was highest in the LCa group mainly during the first two weeks of the experience.

Another proposed mechanism is the decreased availability of energy when Ca and fat are provided jointly. The high CaI could increase fecal excretion of fat, presumably via the formation of insoluble Ca fatty acid soaps in the gut or by binding of bile acids, which impairs the formation of micelles [27]. The low excretion of fat observed in the LCa group did not modify fat absorption percentage, though it may have induced a slight increase in fat availability, which throughout the experience, could have caused a rise in fat accrual. In addition, when fat is absorbed, it enters the blood circulation in the form of intestinally derived TGL-rich lipoproteins, that is, chylomicrons [27]. Thus, if Ca partly inhibits fat absorption, a decrease in TGL could be expected. Conversely, TGL would increase with a diet supplying a low amount of Ca. Although our obese rats spontaneously develop hypertryacilglyceridemia, the highest levels of TGL were observed in the LCa group.

Hyperinsulinism and insulin resistance are characteristic features of obesity consistent with a decrease in insulin receptor activity or post-receptor defects [28]. The increment in the insulin/glucose ratio reflects fat-induced insulin resistance which, according to the severity of the obesity and the rise in hypertriglyceridemia, could contribute to a higher body fat synthesis and fat storage [28]. In the present study, in addition to exhibiting the highest levels of TGL, the LCa group also showed the highest fasting levels of glucose, insulin, and insulin/glucose ratio. As mentioned above, the strain of obese rats used herein spontaneously develops hypertryacilglyceridemia and insulin resistance after puberty. Both the latter were found to increase concomitant to an increase in weight gain rate, following a decrease in the Ca content in the diet.

The negative correlation between dietary Ca intake and changes in BW or lipid content found in the present study is in agreement with several [1, 5, 20, 29–31], though not all [25], previous reports analyzing the potential effect of Ca on body fatness. The proposed biologic mechanism was related to the energy loss through increased expression of uncoupling proteins or increased lipid oxidation [20]. In addition to the inhibition of basal lipolysis, the mechanism

might involve stimulation of the expression and activity of the fatty acid synthetase (FAS) enzyme by a protein product of the agouti gene. The C-terminal portion of this protein presents a three-dimensional structure that functions as a Ca^{++} channel, allowing its entry into a variety of cells (including adipocytes), and maintaining the intracellular concentration of $[Ca^{++}]_i$ constant [32, 33]. The increment in $[Ca^{++}]_i$ stimulates the expression and activity of FAS and at the same time, increases insulin secretion by the pancreas, resulting in excessive deposition of TGL [2, 20].

Impact on Ca and bone metabolism

Ca bioavailability depends not only on luminal Ca concentration but also on age. According to AIN′93, CaA of normal rats feeding the recommended dietary Ca levels reached the highest levels at weaning and decreased thereafter to reach the lowest values in adult life [32].

All the obese rats studied herein were of similar age and were at the end of the high growth rate period; thus, the level of Ca absorption must have been affected by the total amount of dietary Ca. When Ca absorption was expressed in mg/day, as total CaI minus total FCa, the low Ca group reached the lowest level and no differences were observed between the normal and high Ca groups. Nevertheless, the percentage of CaA was similar in the low and normal Ca diets, and as the amount of Ca available in the intestine increased, the %CaA decreased.

Urinary Ca excretion was very low in all groups (0.7–1.3 mg/day), and the amount of absorbed and retained Ca was therefore similar. This finding may partly explain the bone mass results observed in the LCa and HCa groups when compared with the NCa group. Indeed, the lowest supply of dietary Ca limited the level of total body ash content and total skeleton BMC normalized by BW observed in the LCa group. Conversely, the highest dietary Ca content increased the supply of Ca without improving Ca bioavailability because, although ash content was similar, the BMC/BW ratio remained lower than in the NCa group. The latter findings suggest that a plateau value exists above which an increased CaI value does not seem to have any additional effect. The dietary P level, which was the same in the three experimental diets, may have been a limiting factor in reaching higher levels of bone mass in the HCa group.

P is not considered a nutritional problem. However, a high Ca/P intake can affect P absorption [34]. Although the absorbed P was not evaluated, the highest total body Ca/P ratio observed in the HCa group contrasts with the observed low bone mass, and it could compensate the relative P deficiency regarding the NCa group. Another important finding suggesting such imbalance in the dietary Ca/P ratio is the lowest value in total body protein content, which suggests impairment in nitrogen retention. It can be thought that the results of the present study may explain the differences found by other researchers between the experimental results in rats/mice and the human epidemiological studies regarding a better antiobesity effect of dairy Ca than that provided by supplements [7, 33, 35].

One limitation of the present work is the relatively short duration of the study, which focuses on the interrelationship between low CaI and fat mass accrual in a growing rodent model. Further studies should be conducted to confirm whether the observed results can be extended to an adult rodent model. Another limitation is that P absorption, a parameter that could have clarified the findings related to bone mass in the HCa group, was not measured.

Conclusion

Although further research is necessary, low Ca consumption in genetically obese IIMBβ rats during the growth period may modulate BW inducing changes in several lipid metabolism parameters, which could lead to an increase in body fat.

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Conflict of interest The authors declare that there is no conflict of interest associated with this manuscript.

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