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Regular and Long-Term Effects of a Commercial Diet on Bone Mineral Density

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Abstract: (1) Background: Although the effects of diets used worldwide, such as the Mediterranean diet, have been repeatedly studied, the effects of diet plans developed by national nutritionists are unknown. Our cross-sectional study aimed to assess the effects of the commercial Fitlap diet plan, widely used among Estonians, on bone mineral density (BMD), while considering other influential factors (physical activity, body composition, and macro- and micro-nutrients). (2) Methods: A total of 68 women participated (followers of Fitlap diet—FDF, $n = 34$; age-matched controls, $n = 34$). Body composition, bone mineral density (BMD), including the whole body (WB) and areal BMD from the femoral neck (FN) and lumbar spine (LS), and blood micro-nutrient levels were measured. The menu analysis was based on dietary recalls. (3) Results: The Fitlap diet contains significantly more calcium ($p < 0.001$) and magnesium ($p = 0.007$). FDF consume more fiber (coef. 6.49; $p < 0.001$) and protein (coef. 20.12; $p < 0.001$), which influences fat-free mass (coef. 3674.8; $p = 0.008$) and vitamin B12 blood values (coef. 184.98; $p < 0.001$). The only influencing factor of WB BMD, LS, and FN aBMD was fat-free mass (coef. in all locations 0.000009; and $p = 0.015$; $p = 0.015$; $p = 0.01$, respectively). (4) Conclusions: Fitlap is an example of a commercial diet plan that has no negative effects on bones.

Keywords: dietary plan; bone mineral density; hidden hunger; vitamin D; calcium; magnesium

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1. Introduction

Dieting, mainly for weight reduction and a healthy lifestyle, is common today, even among young people [1]. The effects of several internationally known diets (Atkins style diets, Mediterranean diet, and South Beach diet) on weight loss have been studied [2]. At the same time, diet plans developed by various national nutritionists and fitness specialists have become increasingly popular, but there are no detailed data on their effects and nutrient availability. The commercial Fitlap diet plan [3] has been offered since 2015, and currently, about 160,000 adult women in Estonia have used it. Ninety percent of followers aim for weight loss, and although the diet is based on national dietary recommendations, the carbohydrate content of the menu is lower (ca. 20%), and the protein content is higher (40%). According to the commercial Fitlap program manual, each recipe (1000+ recipes) is suitable for every meal due to the similar energy value and nutrient ratio. Vegetables with a caloric content of less than 20 kcal per 100 g are covered by the so-called free reserve and can be eaten indefinitely during a meal. These include, but are not limited to, spinach, pumpkin, tomato, cucumber, bell pepper, and kohlrabi. It is recommended to eat at least 400 g per day of the mentioned vegetables. There are no consumption restrictions on water and unsweetened coffee and tea. In addition, it is recommended that high-fiber foods (such as cereals and fruits or berries) be eaten at least once a day and that red fish be consumed at least twice a week. If necessary, the daily caloric intake can be slightly higher or lower, and the additional energy needs of pregnant and lactating mothers are also considered.

The necessary energy is often obtained from these so-called conscious diets, but there is a lack of micro-nutrients—vitamins and minerals (hidden hunger) [4]. These conditions are often undiagnosed because hidden hunger is not visible and can occur even in overweight or obese people [5]. Most Estonian young people do not follow the national dietary recommendations; for example, 93% of children are deficient in vitamin D, 65% in vitamin B2, 70% in calcium, and 50% in magnesium [6]. Families with financial difficulties are likely to be at risk of nutrient deficiency. Estonian families receiving minimum wage have difficulty reaching the recommended nutrient intake for vitamin D and iodine, and especially mothers for iron, folate, and calcium [7].

Many of the aforementioned vitamins (vitamin D and B12) and minerals (calcium and magnesium) are also associated with bone mineral density (BMD) [8]. Although low BMD has been considered more of a problem in older women [9], BMD deficiencies have recently been reported in younger people [10,11]. In addition to nutrition [12], age, and sex [13], bone density is affected by, among other things, physical activity (PA) [14] and alcohol consumption [15]. Since bone loss does not cause pain before osteoporosis and fractures occur [16], people are often unaware of their bone health. Areal BMD is the most consistent predictor of osteoporotic fracture [17] and is not usually determined in young, healthy adults.

As the number of fractures increases over time [18], fractures also increase health care costs [19]; therefore, it is important to assess how different dietary plans affect the availability of nutrients related to bone density. Our cross-sectional study aimed to assess the effects of the commercial Fitlap diet plan, widely used among Estonians, on BMD, while considering influential factors such as physical activity (PA), body composition (fat mass and fat-free mass), and macro- (carbohydrates, fibers, fat, protein) and micro-nutrients (vitamins D and B12; calcium and magnesium).

2. Materials and Methods

This prospective cross-sectional study was a part of a larger study conducted in June 2021 at Tartu Health Care College (THCC). It is known that June is usually one of the sunniest in Estonia and thus it can affect the value of vitamin D. All procedures were reviewed and approved by the Medical Ethics Committee of the University of Tartu (Estonia; protocol no. 340/T-2, 19 April 2021).

2.1. Participants

The inclusive criteria were: (1) being followers of the Fitlap diet (FDF) for at least three years, and (2) the same number and the same age of controls, who did not follow dietary plans and special diets (i.e., HCG, Dunkan, Atkins, vegetarianism, etc.). The exclusion criteria were menopause, diagnosis of chronic diseases affecting the bones, and absence recurrent fractures. The number of pregnancies, the use of hormonal contraceptives, and smoking were not studied. The study invitation was forwarded to the target group through a responsible employee at Fitlap and on the Facebook page for THCC. Those interested in the study contacted the lead investigator, with whom a time for the study was agreed, and a written informed consent form was signed. A total of 258 women participated in the study, 34 of them FDF; 34 were selected randomly as an age-appropriate control group for adherence to this study's Fitlap diet plan.

2.2. Dietary Assessment and Physical Activity

The questionnaire was compiled by the respondents (general data, dietary recall, chronic skeletal diseases; consumed additional calcium, magnesium, vitamin D, and B12 for more than 60 days of last year, PA). Furthermore, a blood test and body composition analysis (incl. BMD) were performed.

Energy and nutrient intake were assessed using the average of three 24 h dietary recalls (incl. two weekdays and one weekend day). Before the study, participants were instructed to maintain their usual dietary habits [20] and everyday physical activities. The Nutridata System for Research (National Institute for Health Development, Estonia) [21] analyzed data collected through dietary recalls. The dietary analysis included: energy, carbohydrates (incl. fibers), proteins, fats, vitamins D and B12, calcium, and magnesium. The PA level was assessed using The International Physical Activity Questionnaire—Short Form (IPAQ-SF, validated in Estonian) [22], which questions three specific types of activity undertaken during the previous 7 days: vigorous PA (VPA = 8.0 metabolic equivalent—METs), moderate PA (MPA = 4.0 METs), and low PA (LPA = 3.3 METs). According to the official IPAQ guidelines [22], each item (vigorous intensity, moderate intensity, and walking) was summed to estimate the total time spent engaged in PA per week.

2.3. Body Composition

Body height was measured using a Martin metal anthropometer to the nearest 0.1 cm, according to the standard technique. Body mass was measured with minimal clothing to the nearest 0.05 kg with an electronic medical scale (A&D Instruments, Abingdon, UK) and body mass index (BMI) calculated as body mass (kg) divided by body height squared (m^2).

Body composition was determined at the Institute of Sport Sciences and Physiotherapy of the University of Tartu using an X-ray bone densitometer or DXA device (Lunar). The procedure was performed by an institute employee who received the appropriate training. The subject was dressed in light clothing and lying on her back; the device scanned her entire body. The radiation level to the body was low and equal to one day's dose of cosmic radiation during the procedure. Body fat percentage (%), fat-free mass (FFM), fat mass (FM), whole-body (WB) BMD (g/cm^2), and areal bone density (aBMD; g/cm^2) directly from the femoral neck (FN) and lumbar spine (LS) L1–L4 were determined. Coefficients of variation (CVs) for BMD were less than 2%. The International Osteoporosis Foundation recommends using T scores in those aged 20–50 and suggests using a T score of <-2.5 to define osteoporosis [23].

2.4. Blood Analysis

Subjects were asked not to eat for 2 h prior to the sample collection. Blood for analysis (2×3.5 mL with clot activator and gel) was taken by a registered nurse. The serum was separated by centrifugation for clinical chemistry analyses and stored at -20 °C for one month until analysis. The remaining biological material was destroyed by autoclaving. Assays were performed in the THCC laboratory with a fully automated clinical chemistry analyzer Cobas e411 (Roche Diagnostics International AG, Rotkreuz, Switzerland) for the determination of serum vitamins D and B12 and Cobas c111 (Roche Diagnostics International AG, Rotkreuz, Switzerland) for serum magnesium and calcium concentrations. It is the equipment of recognized companies and accuracy was guaranteed by calibration and quality control.

All study participants received feedback on their laboratory and bone density responses and normative parameters from the lead investigator. If the results showed anomalies, this was also considered, and the subject was advised to consult their family doctor for further analysis.

2.5. Statistical Analysis

For statistical analysis, the software programs Sigma Plot for Windows version 11.0 (GmbH Formation, Erkrath, Germany), and R 2.6.2 (A Language and Environment) [24] were used. For descriptive statistics, median and IQR were reported. Continuous data and proportions were compared by *t*-test or Mann–Whitney test and chi-square or Fisher exact tests. Firstly, the influence of the Fitlap diet on macro- and micro-nutrients, PA, and body composition were calculated using univariate linear regression analyses. Secondly, the influence of additionally used calcium, magnesium, and vitamins D and B12 on blood and bone parameters were calculated. Thirdly, the following risk factors for WB BMD, L1–L4 aBMD, and FN aBMD were studied in separate linear regression models: belonging to the FDF group, energy, macro-nutrients (carbohydrates, fiber, fat, protein) in diet, alcohol consumption, PA (vigorous, moderate, low), and body parameters (body mass, height, fat mass, fat-free mass, fat%). All variables significant in the univariate logistic regression model at a *p*-value of <0.08 were included in the multivariate logistic regression model. Subsequently, the variables were removed according to the order of insignificance until only variables with *p*-value < 0.05 were retained. Only BMI (excluding height and mass) and fat mass instead of fat mass % were included to avoid collinearity.

3. Results

3.1. Demographic Data and Body Composition (incl. Bone Mineral Density)

A total of 34 healthy adult female FDF aged 22–48 and same-aged controls were enrolled in this study. The FDF were heavier than controls due to fat-free mass; however, there were no differences in BMI or FM. Whole and areal BMD were higher in FDF than controls. According to the WHO [25], 4 (11.8%) FDF and 10 (29.4%) controls had an osteopenia (T-score −1 to −2.5 SD), and 1 control group member had osteoporosis (<−2.5 SD). Participants with osteopenia consumed additional vitamin D (n = 9), calcium (n = 1), and magnesium (n = 1); participants with osteoporosis consumed vitamin D and calcium. None of the participants were previously aware of their bone health. The main characteristics and body composition parameters are presented in Table 1 and BMD (incl. aBMD) in Figure 1.

Table 1. Demographic data and body composition (presented as median values with quartiles).

n =	All Participants 68	FDF 34	Controls 34	*p* =
Age (year)	37; 31.5–42.5	36.5; 31–43	37; 32–42	NS
Body mass (kg)	70; 62.8–85.3	73.4; 68.0–84.0	66; 59.0–87.0	0.048
Body mass index (kg/m^2)	25.2; 22.3–28.2	26.4; 23.7–28.3	23.2; 20.5–28.1	NS (0.053)
Height (cm)	169.3; 165–172	170.0; 165–172	169.0; 165–173	NS
Fat mass (kg)	24.0; 18.8–33.9	27.2; 20.0–31.5	22.0; 18.4–35.5	NS
Fat-free mass (kg)	43.5; 40.1–48.0	46.0; 42.9–48.2	41.3; 38.5–46.2	0.008
Fat mass%	35.3; 31.0–40.6	35.6; 30.6–40.5	35.0; 31.8–40.6	NS

FDF—Fitlap diet followers; NS—not significant.

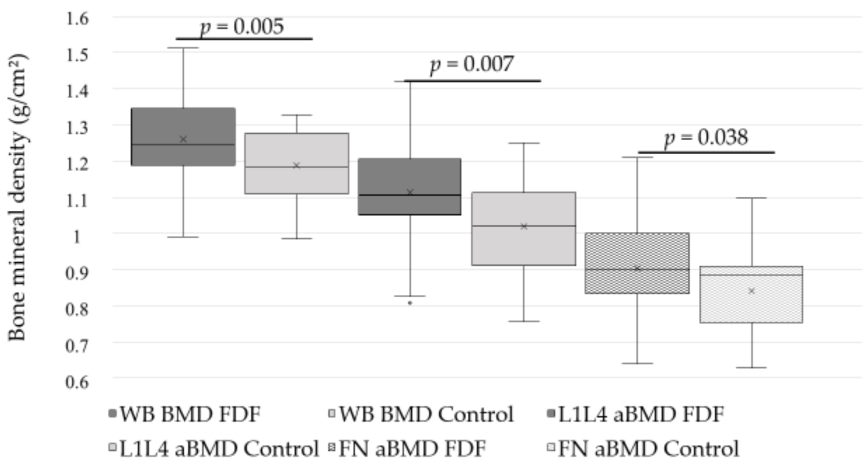

Figure 1. Whole BMD and areal BMD in Fitlap diet followers (FDF) compared with controls (BMD—bone mineral density; aBMD—areal bone mineral density; WB—whole-body; L1L4—lumbar spine (L1–L4); FN—femoral neck.

3.2. Effects of Micronutrients and Vitamins (B12 and D) in Food and as Food Additives on Blood Parameters

Energy consumption was similar in the study groups, but FDF consumed protein and fiber significantly more than the controls (Table 2). The micro-nutrient levels in the blood of Fitlap diets did not differ significantly (except for vitamin B12). Based on the menu analysis, it can be said that the Fitlap diet menu contains significantly more calcium and magnesium. Considering the values recommended for Estonians by Synalab [26] (serum 25-hydroxyvitamin D 25(OH)D > 75 nmol/L, vitamin B12 150 pmol/L, calcium > 2.1 mmol/L, and magnesium > 0.66 mmol/L), the blood values were lower accordingly: 2 vs. 0 for calcium in the FDF group and controls; for magnesium 1 vs. 1, and vitamin D 17 vs. 18. Furthermore, there were significantly more women in the FDF group who engaged in regular vigorous PA.

Table 2. Macro- and micro-nutrients in diet and blood that affect bone mineral density and physical activity (presented as median values with quartiles or n with %).

	All Participants	**Fitlap**	**Controls**	
n =	**68**	**34**	**34**	***p* =**
Energy and macro-nutrients in diet				
Calories (kcal)	1821.4; 1579.4–1944.6	1840.5; 1627.6–2034.0	1741.0; 1507.5–1940.7	NS
Carbohydrates (g)	203.0; 170.7–242.0	215.1; 175.6–247.8	198.6; 158.1–216.5	NS
Fiber (g)	20.1; 16.6–25.6	23.9; 18.5–28.9	18.2; 14.3–21.9	0.001
Fat (g)	74.6; 63.5–86.9	71.6; 63.5–83.1	77.6; 64.1–91.5	NS
Protein (g)	78.7; 64.9–92.7	85.0; 78.6–96.8	65.9; 55.4–80.1	0.001
Alcohol (g)	0.0; 0.0–6.0	0.0; 0.0–9.5	0.0; 0.0–5.0	NS
Micronutrients in diet				
Calcium (mg)	799.0; 261.5–971.4	916.8; 674.4–1089.5	668.2; 518.6–820.7	<0.001
Magnesium (mg)	319.2; 269.5–380.8	335.5; 306.0–394.2	291.2; 227.2–375.6	0.007
Vitamin D (μg)	3.3; 1.8–5.1	3.4; 1.9–5.4	2.9; 1.7–4.8	NS
Vitamin B12 (μg)	4.5; 3.7–5.5	4.5; 3.9–5.4	4.6; 3.3–5.8	NS
Micronutrients in blood				
Calcium (mmol/L)	2.33; 2.28–2.38	2.33; 2.28–2.38	2.33; 2.27–2.38	NS
Magnesium (mmol/L)	0.82; 0.78–0.84	0.82; 0.79–0.84	0.82; 0.77–0.84	NS
Vitamin D (nmol/L)	73.7; 58.5–94.0	75.2; 61.6–93.5	73.3; 55.4–98.5	NS
Vitamin B12 (pg/mL)	446.1; 370.8–572.8	538.2; 428.7–683.3	396.0; 359.1–451.3	0.00
Physical activity				
Vigorous (%)	2; 2.9	0; 0.00	2; 5.9	NS
Optimal (%)	35; 51.5	14; 41.2	21; 61.8	NS
Very active (%)	31; 45.6	21; 61.8	11; 32.4	0.028
Food additives consumption *				
Vitamin D (n; %)	38; 55.9	21; 61.8	20; 58.8	NS
Complex vitamin B (n; %)	6; 8.8	3; 8.8	3; 8.8	NS
Calcium (n; %)	1; 1.5	0; 0.0	1; 2.9	NS
Magnesium (n; %)	10; 14.7	7; 20.6	3; 8.8	NS

NS—not significant; *—consumption of a food supplement for more than 30 days a year.

The regression analysis results show that FDF consumed more fiber (coef. 6.49; $p < 0.001$) and protein (coef. 20.12; $p < 0.001$); fat-free mass (coef. 3674.8; $p = 0.008$) and vitamin B12 laboratory values (coef. 184.98; $p < 0.001$) were also influenced. Additionally, consumed magnesium decreased the magnesium value in blood (coef. −0.03; $p = 0.05$) and calcium in the diet decreased the calcium value in blood (coef. −0.0002; $p = 0.02$).

3.3. Factors Influencing Bone Mineral Density

The results of the univariate linear regression analysis data, which present the influence of different factors on BMD and aBMD, are presented in Table 3. The univariate logistic regression analysis results show that calcium supplements negatively influenced WB BMD and aBMD.

Magnesium in the blood negatively influenced WB BMD and L1–L4 aBMD, and vitamin D supplements negatively influenced L1–L4 aBMD. The multivariate regression analysis results show that the only influencing factor on WB BMD, L1–L4 aBMD, and FN aBMD was fat-free mass (coef. in all locations 0.000009; and $p = 0.015$; $p = 0.015$; $p = 0.01$, respectively).

Table 3. Parameters influencing bone mineral density (results of univariate regression analysis).

Parameter	Influencing Factor	Coef.	$p =$
WB BMD	Fitlap diet	0.073	0.005
	Height	0.005	0.05
	Body mass	0.003	<0.001
	Body mass index	0.009	<0.001
	Fat mass	3.416	0.005
	Fat-free mass	1.062	<0.001
	Calcium supplement	−0.243	0.026
	Calcium in diet	1.114	0.015
	Magnesium in blood	−0.381	0.05
L1–L4 aBMD	Fitlap diet	0.094	0.007
	Height	0.010	0.005
	Body mass	0.006	<0.001
	Body mass index	0.017	<0.001
	Fat mass	6.508	<0.001
	Fat %	0.008	0.003
	Fat-free mass	1.812	<0.001
	Vitamin D supplement	−0.070	0.05
	Calcium supplement	−0.315	0.033
	Calcium in diet	1.585	0.010
	Magnesium in blood	−0.514	0.05
Femoral neck aBMD	Fitlap diet	0.062	0.038
	Height	0.007	0.018
	Body mass	0.005	<0.001
	Body mass index	0.012	<0.001
	Fat mass	4.559	<0.001
	Fat %	0.006	0.008
	Fat-free mass	1.279	<0.001
	Calcium supplement	−0.248	0.044
	Calcium in diet	1.450	0.005

BMD—bone mineral density, aBMD—areal bone mineral density.

4. Discussion

Our study is the first in Estonia to discuss the availability of micro-nutrients in a widely used commercial diet plan and its effect on bone density. The study results clearly indicate that a regulated commercial diet, i.e., regular adherence to a long-term diet (>3 years) is important for bone health. Our study showed that BMD is mainly affected by fat-free mass in adult women. However, micro-nutrients that affect bone density, such as calcium, magnesium, and vitamin D, should be obtained by food and not as a food supplement.

Overall, the results for bone density are concerning—22% of the entire study group (incl. 32.4% of controls) suffer from bone loss (osteopenia or osteoporosis) despite their relatively young age. It can also be assumed that young adults can no longer achieve so-called healthy BMD, likely due to the widespread inactivity [27] and unbalanced diet [28] among children today. The effect of obesity on BMD during growth is still unclear [29]; however, it has been found that the BMI's positive effects on bone are at least partly mediated through greater fat-free mass [30,31]. As muscle mass increases, bone is exposed to increased mechanical loads and responses through the modeling and remodeling processes, leading to osteogenesis [32]. Weight-bearing PA has beneficial effects on bone mineral accretion [33], and overweight (including obese) children engage in less moderate—vigorous PA [34] and spend more time in screen-focused sedentary behaviors [35]. In addition to inactivity, today's young people often drink plenty of carbonated soft drinks instead of milk, which detrimentally affects the accumulation of bone minerals [36]. Obese children are also deficient in vitamin D [37], which reduces the absorption of already low calcium [38]. Bone formation is most intense in adolescence; for example, FN and hip aBMD are reached between the ages of 16 and 19 [39], and childhood obesity is worldwide [40]. However, it can be expected that BMD problems will become more common in young adults over the years.

Although it has been found that PA can play an important role in reducing fractures even in postmenopausal ages [41], our study results did not show the effects of different PA levels on bone. However, since our results did show that fat-free mass is the major factor influencing BMD, as was found by other authors [42], and due to PA, we cannot claim that PA does not affect BMD. The assessment of physical activity on the basis of the IPAQ questionnaire is rather subjective and thus we cannot be completely sure of the actual level of physical activity of the subjects.

Although the Fitlap diet contains significantly higher levels of calcium and magnesium in the diet than control group, the differences of these microelements in the blood did not emerge. It is anticipated that organisms without durative pathology catch several times after eating to balance the blood values. Previously, it has been considered that calcium absorption decreases with age, and important independent positive predictors are dietary fat and serum estradiol and 1,25-dihydroxyvitamin D3 [43]. Intestinal calcium absorption increases during a high-protein diet [44] and dietary protein positively influences calcium metabolism that influences bone health and is associated with molecular, cellular, and endocrine bases of the interactions [45]. Also, the multiple factors additional to dietary intake affecting magnesium status, such as high pH (<7.4) in ileum, gender (young women have better magnesium retention than young men), and weight (obese, BMI $\geq$ 30) have been shown to have lower magnesium consumption and reduced magnesium status. Serum changes can also be influenced by albumin levels [46], but the results of our study show a high serum value negative effect to bone density. Increased dietary fiber intake does not affect magnesium status but can increase magnesium excretion in feces [46].

In recent decades, the need for calcium has been repeatedly discussed, and as recommended amounts are higher than what people normally receive from their diet, the supplemental calcium intake is widespread [47]. Our results confirm that dietary calcium intake is insufficient compared with the recommended amount [48], but serum calcium levels are predominantly within the reference range. Dietary calcium has a significant positive effect on both the WB and areal BMD, whereas calcium supplements' effects on bone are the opposite. In their meta-analysis, Bischoff–Ferrari et al. [49] reached a similar result, which showed an increased risk of hip fractures with calcium supplementation. This result can be explained by Reid et al. [47], based on a literature review, who suggest that calcium supplements act as weak anti-resorptive agents, reducing bone turnover and not producing cumulative benefits in terms of bone mass.

Women often consume only half of the recommended amount but do not need calcium supplementation, mainly because supplemental calcium does not play a significant role in preventing and treating osteoporosis. There are several risks associated with consuming supplemental calcium. One concern is that this mineral does not accumulate in bone alone [50], and there is a significant increase in the risk of cardiovascular diseases with high supplemental calcium intake [51]. However, the safety of consuming calcium supplements is still unclear, and our results confirm that the need for calcium can still be obtained through food [52].

Vitamin D is an essential nutrient that plays a fundamental role in maintaining serum calcium and bone health throughout life [8]. Since our main source of vitamin D is exposure to sunlight [53], its deficiency is common in Estonians year-round [54]. Although Kull et al. [54] consider the recommended level too high for Estonians, our study's serum vitamin D level was still low, despite being conducted in June, which is usually one of the sunniest months in Estonia. Although the positive effects of vitamin D supplementation on BMD have been found repeatedly in elderly women [55,56], the benefits of vitamin D supplementation in younger women are not clear [8].

Our study cannot confirm the positive effects of vitamin D supplementation. Although the statistical analysis did not show statistical significance, we can assume that vitamin D supplementation in a larger study group would negatively affect trabecular bone (L1–L4 aBMD). Although the recent meta-analysis does not support a major treatment benefit of vitamin D for osteoporosis [57], it is clear that severe vitamin D deficiency can lead to multiple avoidable illnesses and inadequate vitamin D status impacts on health care costs [58].

The shortcomings of our research are the relatively small sample and the cross-sectional research method. In order to ensure the homogeneity of the study group, smokers, and long-term users of hormonal drugs (including contraceptives) should also be excluded. An examination of bone turnover markers would certainly be necessary in the following study. However, significantly different methods were used (menu analysis, laboratory studies, DXA). Although the menu data analysis may not accurately reflect everything eaten and its fulfillment is still subjective, it provides an overview of the situation, which appears credible by the results. Unfortunately, there are no data on how long the subjects have followed the commercial Fitlap diet and how physically active they were at school age.

5. Conclusions

Regular adherence to a balanced commercial diet benefits micro-nutrient intake (calcium and magnesium) and BMD. However, micro-nutrients that affect bone density, such as calcium, magnesium, and vitamin D, should be obtained as food and not as a food supplement. However, it must be remembered that a rational diet is a varied diet that meets a person's need for energy and nutrients. Fitlap is an example of a commercial diet plan that has no negative effects on bones. Thus, it is necessary to teach a balanced diet and develop eating and exercise habits from early school age, because according to Movassagh [59], healthy dietary habits established during childhood and adolescence may continue into adulthood.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data is contained within the article.

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Conflicts of Interest: The authors declare no conflict of interest.

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