Calcium to magnesium intake ratio and non-alcoholic fatty liver disease development: a case-control study

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide. Adoption of sedentary life style and westernized diet are shown to be associated with development of NAFLD. Since previous studies suggested that calcium (Ca) to magnesium (Mg) ratio intake is associated with some chronic diseases including dyslipidemia and insulin resistance, we designed this study to find any possible association between this ratio and NAFLD development.

Methods: The NAFLD was diagnosed using Fibroscan according to a CAP cut-off value of 263 dB/m. Dietary intakes of one hundred and ninety-six patients with incident NAFLD diagnosis, and eight hundred and three controls without NAFLD were assessed using a valid food frequency questionnaire (FFQ). Dietary nutrients were calculated using Nutritionist IV software.

Results: Age of the study population (57 % female) was 43.2 ± 14.1 years. In addition, energy-adjusted daily calcium to magnesium intake ratio was 2.34 ± 0.57 and 2.73 ± 0.69 for control and case groups, respectively. In the multivariable-adjusted model, after adjustment for potential confounding variables; including, age, gender, BMI, alcohol consumption, smoking, diabetes, physical activity, energy, dietary fiber, carbohydrate, fat, and protein intakes, participants in the third (Q3) and fourth (Q4) quartile of Ca/Mg ratio intake had a greater development of incidental NAFLD compared to the lowest quartile (Q1) [(OR = 2.86; 95 % CI: 1.20–6.81), (P-value = 0.017) and (OR = 5.97; 95 % CI: 2.54–14.01), (P-value < 0.001) for Q3 and Q4 compared to the Q1, respectively]. Moreover, energy-adjusted Ca to Mg intake ratio was positively correlated with plasma level of ALT (r = 0.18; P = 0.01); contrarily, it had no correlation with plasma levels of AST.

Conclusions: The current study revealed that higher dietary Ca to Mg intake ratio is associated with a greater development of NAFLD. Further interventional studies are needed to confirm the causal relationship of the Ca/Mg ratio intake and development of NAFLD.
Background
Non-alcoholic fatty liver disease (NAFLD) includes the entire spectrum of fatty liver disease in patients without significant alcohol consumption, ranging from fatty liver to steatohepatitis to cirrhosis [1]. NAFLD is one of the most common causes of liver cirrhosis and liver cancer [2–4]. The mortality rate of liver cirrhosis and cirrhosis-related diseases such as liver cancer has increased over the past 35 years worldwide [5]. Obesity, type 2 diabetes, overdosage, and exposure to the toxic substances constitute the etiology of NAFLD [6–8]. Patients with metabolic syndrome are much more likely to develop NAFLD [9, 10]. It has been estimated that NAFLD will be one of the leading causes of liver disease and its consequent mortality by 2030, along with the obesity epidemic [11]. There is also a population so called “lean NAFLD” [12]. It has been shown that weight reduction is also beneficial in this population [13]. The first line in the treatment of NAFLD is dietary interventions and reducing central obesity [14]. Although the pathogenic role of macronutrients in NAFLD and obesity are clear, the contribution of micronutrients in the pathogenesis of NAFLD has garnered less attention than that of obesity [15]. Overall, micronutrients play an important role in NAFLD pathogenesis [16].

Magnesium (Mg) is an abundant cation in the human body that plays essential roles in multiple physiological pathways such as cellular energy metabolism, inflammation, nucleic acid metabolism, protein synthesis, and electrolyte balance [17]. Serum Mg concentration is strictly controlled; however, hypomagnesemia may occur as a result of increased renal excretion or decreased digestive absorption of magnesium [18]. Hypomagnesemia and subclinical Mg deficiency are associated with osteoporosis, seizure, depression, diabetes mellitus, hypertension, dyslipidemia, and colorectal cancer [19–21]. On the other hand, an observational study found that higher intakes of Mg are associated with a lower development of NAFLD in young adults [22].

Calcium (Ca) is another major mineral that is mainly deposited in bone and plays a key role in various biological processes [23]. Ca and Mg could be regarded as antagonist to each other in various biochemical pathways [24]. Studies show that altered serum calcium is associated with dyslipidemia and insulin resistance [25]. Previous studies on human subjects indicate that a high Ca intake may affect the absorption rate of Mg, which in turn, can be associated with an increased risk of several diseases [26–29]. Taking all these facts into account, it is plausible to consider the intake ratio of calcium to magnesium as a potential contributor to NAFLD pathogenesis [28].

Due to the novelty of the idea, we decided to examine the possible relationship between calcium to magnesium intake ratio and development of NAFLD in this study.

Materials/subjects and methods
This case–control study was conducted at two clinics in Tehran province of Iran. The study included 196 cases with NAFLD and 803 controls. These participants were selected with the convenience sampling procedure. We conducted this case-control study on patients with recently diagnosed NAFLD and age-matched controls subjects from the same clinic. The absence of hepatic steatosis in individuals in the control group was determined using the ultrasound exam. The presence of NAFLD in our cases was confirmed by a gastroenterologist; moreover, the inclusion criteria included having a Controlled Attenuation Parameter (CAP) score of more than 263, fibrosis score more than 7, determined by the Fibroscan device, and the intake of alcohol less than 20 gram/day in women, and less than 30 gram/day in men. Fibroscan is an ultrasound device calibrated to measure hepatic steatosis levels producing an index called CAP; CAP score indicates the level of infiltration of fat in hepatocytes and has sensitivity and specificity ranged between 78 and 100% [30]. All tests were performed by one operator with Fibroscan (EchoSens, Paris, France) device. Patients fasted for at least 3 h before the test. Patients were lying on the bed for at least 3 min before the test. The probes were calibrated before starting work. The other secondary causes of hepatic fat accumulation such as long-term use of a steatogenic medication (e.g., mipomersen, lomitapide, amiodarone, methotrexate, tamoxifen, corticosteroids, valproate and anti-retroviral medicines), or Hepatitis C (genotype 3) or Wilson disease or lipodystrophy or starvation or abetalipoproteinaemia or Reye syndrome or inborn errors of metabolism were our exclusion criteria [1].

A validated food frequency questionnaire (FFQ) was used to assess the dietary intake of the participants [31]. Additionally, calorie-density of the two minerals (the amount of each mineral in an energy intake of 1000 Kcals) and their ratio were calculated for further analysis.

Baseline characteristics and dietary intakes between two study groups were compared using student t-test for continuous variables and chi-square for categorical variables. We used SPSS (Version 21.0; Chicago, IL, USA) software to conduct the statistical analyses. The study participants were divided into four categories based
onquartiles of daily energy-adjusted calcium to magnesium ratio and the lowest quartile was set as the reference category in order to evaluate the association between energy-adjusted daily calcium to magnesium intake ratio and NAFLD development. We took advantage of ANOVA test to compare the inter-quartile relationships. Odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated using multiple logistic regression analysis. Analyses were adjusted for all known confounding factors. P-value less than 0.05 considered as significant.

Results

Baseline participants’ characteristics in case and control group are shown in Table 1. The participants in case group had higher levels of BMI, fasting blood sugar (FBS), triglyceride (TG), low-density lipoprotein–cholesterol (LDL-c), physical activity (PA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), smoking, diabetes, male, protein, calcium and calcium to magnesium intake ratio and lower levels of high-density lipoprotein–cholesterol (HDL-c), carbohydrate and fat intake (P < 0.05). Baseline characteristics regarding energy-adjusted daily calcium to magnesium intake ratio quartiles are presented in Table 2. Mean ± SD age of the study population (57 % female) was 43.2 ± 14.1 years. In addition, mean ± SD of energy-adjusted daily calcium to magnesium intake ratio was 2.34 ± 0.57 and 2.73 ± 0.69 for control and case groups, respectively.

Inter-quartile analyses revealed that participants with higher daily calcium to magnesium intake ratio had significantly higher prevalence of smoking (P < 0.001), higher levels of BMI (P = 0.006), and higher levels of LDL-c (P = 0.033) compared to those with lower daily calcium to magnesium intake ratio. Significant differences were also detected regarding dietary intakes of energy (P = 0.024), carbohydrate (P < 0.025), fat (P = 0.042), and dietary fiber (P = 0.001) when inter-quartile data were analyzed. Compared by the same criteria, no other statistically significant differences were found.

Firstly, we evaluated the associations of energy adjusted calcium and magnesium intake with the development of NAFLD (Tables 3 and 4). Regarding to the energy adjusted calcium intake, in the final model adjustments for potential confounding variables; including, age, gender, BMI, alcohol consumption, smoking.

Table 1 Baseline participants’ characteristics in case and control group

| Characteristics               | Case (n = 196) | Control (n = 803) | P value |
|-------------------------------|---------------|------------------|---------|
| Age (years)                   | 42.3 ± 11.9   | 43.5 ± 14.5      | 0.214   |
| Male (%)                      | 51.5          | 41.0             | 0.007   |
| Body mass index (kg/m²)       | 35.7 ± 10.6   | 27.7 ± 4.5       | < 0.001 |
| Fasting blood glucose (mg/dl) | 108.5 ± 37.6  | 90.2 ± 29.4      | < 0.001 |
| Triglycerides (mg/dl)         | 176.0 ± 117.9 | 132.0 ± 81.8     | < 0.001 |
| Total cholesterol (mg/dl)     | 185.7 ± 52.8  | 177.5 ± 38.9     | 0.118   |
| High Density Lipoprotein–Cholesterol (mg/dl) | 41.9 ± 16.1 | 47.7 ± 10.5      | 0.001   |
| Low Density Lipoprotein–Cholesterol (mg/dl) | 120.9 ± 41.6 | 104.0 ± 31.9     | < 0.001 |
| Physical activity (MET(hour/week)) | 31.0 ± 3.2 | 34.2 ± 3.1       | < 0.001 |
| Alanine aminotransferase (U/L) | 55.71 ± 8.27 | 20.49 ± 7.59     | < 0.001 |
| Aspartate aminotransferase (U/L) | 33.86 ± 23.69 | 22.14 ± 7.72    | < 0.001 |
| Current alcohol usage (%)     | 12.8          | 9.1              | 0.07    |
| Current smoking (%)           | 89.7          | 18.8             | < 0.001 |
| Diabetes (%)                  | 16.6          | 6.8              | < 0.001 |
| Energy (Kcal/d)               | 2757 ± 961.1  | 2804 ± 840.7     | 0.499   |
| Protein (% of energy)         | 15.8 ± 2.9    | 14.1 ± 2.3       | < 0.001 |
| Carbohydrate (% of energy)    | 58.2 ± 6.3    | 59.8 ± 13.3      | 0.014   |
| Fat (% of energy)             | 29.2 ± 5.3    | 33.8 ± 5.7       | < 0.001 |
| Total dietary fiber (g/d)     | 46.29 ± 19.07 | 55.69 ± 46.11    | 0.08    |
| Magnesium (mg/d)              | 484 ± 155     | 509 ± 173        | 0.09    |
| Calcium (mg/d)                | 1389 ± 458    | 1114 ± 390       | < 0.001 |
| Calcium to Magnesium ratio    | 2.79 ± 0.68   | 2.34 ± 0.57      | < 0.001 |

Quantitative data are presented as mean ± SD and qualitative data reported as percent

* Independent sample t-test and chi-square or Fisher exact test for quantitative and qualitative parameters, respectively
### Table 2: Baseline participants' characteristics across quartiles of energy-adjusted daily calcium to magnesium ratio intake

| Characteristics                      | Q1 (n = 250) | Q2 (n = 249) | Q3 (n = 250) | Q4 (n = 250) | P value* |
|--------------------------------------|--------------|--------------|--------------|--------------|---------|
| Age (years)                          | 45.1 ± 13.7  | 41.9 ± 14.3  | 43.5 ± 14.1  | 43.3 ± 14.3  | 0.16    |
| Male (%)                             | 42.4         | 45           | 46.8         | 38           | 0.21    |
| Body mass index (kg/m²)              | 28.7 ± 5.2   | 27.8 ± 5.3   | 28.4 ± 5.8   | 29.1 ± 6.7   | 0.006   |
| Fasting blood glucose (mg/dl)        | 94.4 ± 34.6  | 92.9 ± 34.1  | 89.8 ± 30    | 92.3 ± 27.1  | 0.44    |
| Triglycerides (mg/dl)                | 139 ± 92.1   | 140 ± 109.6  | 133 ± 71.9   | 131 ± 67.9   | 0.69    |
| Total cholesterol (mg/dl)            | 179 ± 36.3   | 178 ± 44.4   | 175 ± 33.9   | 183 ± 37.1   | 0.33    |
| High Density Lipoprotein-Cholesterol (mg/dl) | 46.7 ± 10.2 | 46.4 ± 11.2  | 47.2 ± 10.3  | 49.1 ± 11.8  | 0.44    |
| Low Density Lipoprotein-Cholesterol (mg/dl) | 104 ± 30.7  | 103 ± 35.6   | 101 ± 29.6   | 107 ± 29.5   | 0.033   |
| Physical activity (MET(hour/week))  | 32.1 ± 4.4   | 34.9 ± 3.3   | 31 ± 3.9     | 33.8 ± 2.1   | 0.22    |
| Alanine aminotransferase (U/L)       | 22.9 ± 23.1  | 23.7 ± 28.6  | 24.2 ± 14.1  | 23.4 ± 19.1  | 0.08    |
| Aspartate aminotransferase (U/L)     | 23.1 ± 10.3  | 23.6 ± 13.6  | 23.5 ± 9.2   | 22.1 ± 8.9   | 0.95    |
| Current alcohol usage (%)            | 9.6          | 10.4         | 9.2          | 10           | 0.97    |
| Current smoking (%)                  | 25.6         | 32.1         | 28.8         | 44.2         | < 0.001 |
| Diabetes (%)                         | 10.8         | 7.6          | 7.6          | 8.9          | 0.53    |
| Energy (Kcal/d)                      | 2712 ± 857   | 2846 ± 815   | 2876 ± 930   | 2758 ± 769   | 0.024   |
| Protein (% of energy)                | 14.8 ± 1.5   | 14.4 ± 3.3   | 15.1 ± 1.7   | 14.9 ± 6.3   | 0.16    |
| Carbohydrate (% of energy)           | 58.6 ± 12.3  | 56.8 ± 10.9  | 54.9 ± 8.2   | 59.1 ± 8.7   | 0.025   |
| Fat (% of energy)                    | 29.4 ± 1.3   | 32.5 ± 2.1   | 30.9 ± 7.6   | 29.1 ± 4.2   | 0.042   |
| Total dietary fiber (g/d)            | 43.6 ± 16    | 45.4 ± 17.6  | 50.1 ± 36.8  | 50.1 ± 21.1  | 0.001   |
| Magnesium (mg/d)                     | 521 ± 178    | 508 ± 154    | 491 ± 167    | 434 ± 130    | < 0.001 |
| Calcium (mg/d)                       | 879 ± 315    | 1106 ± 340   | 1249 ± 419   | 1404 ± 412   | < 0.001 |
| Calcium to Magnesium ratio           | 1.69 ± 0.20  | 2.17 ± 0.11  | 2.54 ± 0.12  | 3.25 ± 0.38  | 0.001   |

Quantitative data are presented as mean ± SD and qualitative data reported as percent

*One way ANOVA test and chi-square or Fisher exact test for quantitative and qualitative parameters, respectively

### Table 3: The development of non-alcoholic fatty liver disease across quartiles of energy-adjusted daily calcium intake

| Energy-adjusted daily calcium intake | Q1(n = 249) | Q2(n = 250) | P-value* | Q3(n = 250) | P-value* | Q4(n = 250) | P-value* |
|-------------------------------------|------------|------------|----------|------------|----------|------------|----------|
| Cases/control                       | 18/231     | 25/225     |          | 49/201     |          | 104/146    |          |
| Range of energy-adjusted Ca         | 135.8 to 347.3 | 347.7 to 407.3 |        | 407.4 to 473.4 |      | 473.9 to 1034.2 |    |
| Model 1 (Ref)                       | 1.42 (0.75–2.68) | 0.27  |          | 3.12 (1.76–5.54) | < 0.001 | 9.14 (5.31–15.71) | < 0.001 |
| Model 2 (Ref)                       | 1.43 (0.75–2.70) | 0.28  |          | 3.34 (1.87–5.96) | < 0.001 | 9.96 (5.73–17.33) | < 0.001 |
| Model 3 (Ref)                       | 1.92 (0.81–4.54) | 0.13  |          | 2.81 (1.70–6.51) | < 0.001 | 6.34 (3.05–10.39) | < 0.001 |
| Model 4 (Ref)                       | 1.99 (0.76–5.17) | 0.15  |          | 3.03 (1.25–7.36) | < 0.001 | 5.41 (3.87–10.87) | < 0.001 |

Data are presented as odds ratio (95 %CI)

*Logistic regression

*crude model

*Adjusted for age and gender

*Additionally adjusted for body mass index, alcohol consumption, smoking, diabetes and physical activity

*Additionally adjusted for energy, dietary fiber, carbohydrate, fat, and protein intakes

The significance level: P < 0.05
diabetes, physical activity, energy, dietary fiber, carbohydrate, fat, and protein intakes, a positive association was detected between dietary calcium intake and the development of NAFLD in the third (Q3) and fourth (Q4) quartile compared to the lowest quartile (Q1) \((OR = 3.03; 95\% CI: 1.25–7.36), \(P\)-value = 0.014) and \((OR = 5.41; 95\% CI: 3.87–10.87), \(P\)-value < 0.001) for Q3 and Q4 compared to the Q1, respectively\) (Table 3). However, analyzes showed a negative association between the energy adjusted dietary magnesium intake and the development of NAFLD in fourth (Q4) quartile compared to the lowest quartile (Q1) \((OR = 0.89; 95\% CI: 1.25–4.78), \(P\)-value = 0.043\) (Table 4).

The data regarding the association between energy-adjusted calcium to magnesium intake ratio and the development of NAFLD is presented in Table 5. In the crude model, a positive association was detected between dietary calcium to magnesium intake ratio and the development of NAFLD in the third (Q3) and fourth (Q4) quartile compared to the lowest quartile (Q1) \((OR = 2.01; 95\% CI:1.21–3.34), \(P\)-value = 0.007) and \((OR = 4.33; 95\% CI:2.68–6.97), \(P\)-value < 0.001) for Q3 and Q4 compared to the Q1, respectively\). In the multivariable-adjusted model, after adjustment for potential confounding variables; including, age, gender, BMI, alcohol consumption, smoking, diabetes, physical

### Table 4
The development of non-alcoholic fatty liver disease across quartiles of energy-adjusted daily magnesium intake

| Energy-adjusted daily magnesium intake | Q1\((n = 250)\) | Q2\((n = 249)\) | \(P\)-value* | Q3\((n = 250)\) | \(P\)-value* | Q4\((n = 250)\) | \(P\)-value* |
|---------------------------------------|----------------|----------------|-------------|----------------|-------------|----------------|-------------|
| Cases/control                         | 70\(\uparrow\)180 | 65\(\uparrow\)185 |             | 55\(\uparrow\)194 |             | 36\(\uparrow\)214 |             |
| Range of energy-adjusted Mg           | 86.31 to 154.40 | 154.43 to 174.70 |             | 174.71 to 196.63 |             | 196.65 to 329.96 |             |
| aModel 1                              | 1 (Ref)         | 1.61 (0.57–2.34) | 0.54        | 1.56 (0.78–3.49) | 0.32        | 1.25 (0.61–5.56) | 0.26        |
| bModel 2                              | 1 (Ref)         | 1.32 (0.75–3.32) | 0.36        | 1.11 (0.65–4.31) | 0.41        | 1.09 (0.74–6.32) | 0.14        |
| cModel 3                              | 1 (Ref)         | 1.88 (0.53–4.53) | 0.24        | 2.02 (0.48–6.21) | 0.34        | 0.93 (0.81–5.70) | 0.07        |
| dModel 4                              | 1 (Ref)         | 1.60 (0.43–2.34) | 0.97        | 0.98 (0.71–3.61) | 0.25        | 0.89 (1.25–4.78) | 0.043       |

Data are presented as odds ratio (95 %CI)

aLogistic regression

bAdjusted for age and gender

cAdditionally adjusted for body mass index, alcohol consumption, smoking, diabetes and physical activity
dAdditionally adjusted for energy, dietary fiber, carbohydrate, fat, and protein intakes

The significance level: \(P < 0.05\)

### Table 5
The development of non-alcoholic fatty liver disease across quartiles of energy-adjusted daily calcium to magnesium ratio intake

| Energy-adjusted daily calcium to magnesium ratio intake | Q1\((n = 250)\) | Q2\((n = 249)\) | \(P\)-value* | Q3\((n = 250)\) | \(P\)-value* | Q4\((n = 250)\) | \(P\)-value* |
|--------------------------------------------------------|----------------|----------------|-------------|----------------|-------------|----------------|-------------|
| Cases/control                                          | 277/223        | 34/215         |             | 49/201         |             | 86/164         |             |
| Range of Ca to Mg ratio                                | 0.95 to 1.97   | 1.97 to 2.36   |             | 2.36 to 2.81   |             | 2.82 to 5.02   |             |
| aModel 1                                               | 1 (Ref)        | 1.30 (0.76–2.23) | 0.33        | 2.01 (1.21–3.34) | 0.007       | 4.33 (2.68–6.97) | < 0.001     |
| bModel 2                                               | 1 (Ref)        | 1.22 (0.71–2.11) | 0.46        | 1.93 (1.15–3.21) | 0.012       | 4.45 (2.75–7.21) | < 0.001     |
| cModel 3                                               | 1 (Ref)        | 1.57 (0.73–3.35) | 0.24        | 2.89 (1.35–6.16) | 0.006       | 5.72 (2.82–11.60) | < 0.001     |
| dModel 4                                               | 1 (Ref)        | 1.82 (0.76–4.37) | 0.17        | 2.86 (1.20–6.81) | 0.017       | 5.97 (2.54–14.01) | < 0.001     |

Data are presented as odds ratio (95 %CI)

aLogistic regression

bAdjusted for age and gender

cAdditionally adjusted for body mass index, alcohol consumption, smoking, diabetes and physical activity
dAdditionally adjusted for energy, dietary fiber, carbohydrate, fat, and protein intakes

The significance level: \(P < 0.05\)
activity, energy, dietary fiber, carbohydrate, fat, and protein intakes, participants in the third (Q3) and fourth (Q4) quartile had a greater development of incidental NAFLD compared to the lowest quartile (Q1) [(OR = 2.86; 95% CI: 1.20–6.81), (P-value = 0.017) and (OR = 5.97; 95% CI: 2.54–14.01), (P-value < 0.001) for Q3 and Q4 compared to the Q1, respectively].

To investigate the relationship between the energy-adjusted calcium to magnesium intake ratio and plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), the Pearson correlation test was conducted. Energy-adjusted calcium to magnesium intake ratio was positively correlated with plasma level of ALT (r = 0.18; P = 0.01); contrarily, it had no correlation with plasma levels of AST.

**Discussion**

In this case control study, we observed that subjects with higher energy-adjusted dietary calcium to magnesium intake ratio had a greater development for incidental NAFLD, independent of confounding factors including age, gender, BMI, alcohol consumption, smoking, diabetes, physical activity, energy, dietary fiber, carbohydrate, fat, and protein intakes.

Recent studies have shown that the impact of nutritional factors on the incidence of NAFLD is more pronounced [32–35]. To the best of our knowledge, there is no study that has examined the relationship between the calcium to magnesium intake ratio and the development of NAFLD; however, calcium and magnesium were separately investigated as potential mediators in the pathogenesis of the disease. Based on consistent evidence, intake of magnesium was inversely associated to the factors related to the risk of insulin resistance [36], and metabolic syndrome, specifically factors such as high fasting glucose level, high waist circumference, and low high-density lipoprotein cholesterol [37, 38]. Lu et al. found that higher intakes of Mg during adulthood is related to a lower risk of NAFLD in middle age [22]. Moreover, in another study, subjects with NAFLD or alcoholic fatty liver were at higher risk of developing magnesium deficiency [39]. However, another study conducted in Canada was not able to find any links between magnesium intake and risk of NAFLD; considering the structural weaknesses in this study, the results might lack enough credibility to be deductible [40].

As mentioned beforehand in the present article, calcium has some biochemical interactions with magnesium. These interactions might have clinical manifestations concerning the pathogenesis of several metabolic disorders. Wenshuai Li et al. who investigated the data related to the Third National Health and Nutrition Examination Survey (NHANES III) follow-up US adults’ cohort [41], found out that the intake of magnesium was associated to an approximately 30% reduced risk of NAFLD and prediabetes, only in subjects who consumed less than 1200 mg/day calcium. Their findings suggested that beneficial effect of magnesium might be attenuated when calcium intake is higher than the amount recommended by Dietary Reference Intakes (DRIs). One of the important mechanisms might be the suppressive effects of high calcium intake on the gastrointestinal absorption and renal reabsorption of magnesium which may alter the excretion of the ion in the feces and urine [24, 42].

As evident by the results of the present study, the Ca:Mg intake ratio might be a useful indicator to observe the combined impact of these two major minerals may exert upon different physiologic, as well as pathologic pathways. USDA food surveys from 1977 to 2007-8 show a rising food Ca:Mg ratio from 2.3 to 2.9 to 2.9–3.5 for all USA adults; these figures might be worthy of concern as they coincide with the rise of several metabolic diseases such as diabetes and colorectal cancer [43]. Furthermore, there is growing evidence indicating that modifications in serum Ca:Mg ratio is associated with some disorders including diabetes and metabolic syndrome [44], breast cancer [45, 46], cardiovascular diseases [47], and higher mortality [29]. Even though the optimal calcium to magnesium intake ratio is yet to be specified, some studies have suggested a 2:1 ratio as the optimum ratio [47]. Consistent with previous studies, our study indicated that participants in the third and fourth quartiles of Ca:Mg intake ratio (ranging from 2.36 to 5.02) had a significantly higher risk of NAFLD as compared to the first quartile.

The regulation of calcium and magnesium homeostasis in the body is interdependent. Calcium-sensitive receptor, also known as CaSR, is responsible to monitor plasma levels of both cations [48].Whenever the plasma level of each of the cations drops, CaSR up regulates the related mechanisms leading to an increase in its blood level, regardless of the other cation concentration in the blood [49, 50]. Furthermore, a drop in serum Mg could also decrease intracellular levels of Mg reducing the cellular Mg–ATP deposits. This may lead to an increase in Ca influx, which, in turn, may upgrade the Ca–ATP level of the cell. Increased intracellular calcium levels have been proposed an underlying mechanism for the pathogenesis of several metabolic and inflammatory disorders such as obesity, metabolic syndrome, diabetes, and NAFLD [45, 51–53].

This is the first observational study that evaluated the relationship between Ca:Mg intake ratio and the development of NAFLD. We were able to conduct the present research on a statistically acceptable sample size of subjects with corresponding socio-economic status. Moreover, the use of top-notch devices to determine the presence or the lack of the disease improved the
Conclusions
The current study revealed that higher dietary calcium to magnesium intake ratio is associated with a greater development of NAFLD. Further interventional studies are needed to confirm the causal relationship between the dietary calcium to magnesium intake ratio and incidental NAFLD.

Abbreviations
NAFLD: Non-alcoholic fatty liver disease; CAP: Controlled Attenuation Parameter; FFQ: Food frequency questionnaire; MET: Metabolic equivalent; BMI: Body mass index; HDL-c: High density lipoprotein; FBS: Fasting blood sugar; TG: Triglycerides; LDL-c: Low-density lipoprotein; PA: Physical activity; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; CaSR: Calcium-sensitive receptor

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Authors’ contributions
A.H. and H.E. conceptualized the study, collected the data, and wrote the manuscript. H. Gh., A.S.T. and H.T. analyzed the data and contributed to drafting of the manuscript. A.H supervised the study. All authors approved the final version of the manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
The study protocol was approved by the ethics committee of the Shahid Beheshti University of medical sciences (ethical code: IR.SBMU.RETECH.REC.1398.588). Written informed consent was signed by all subjects participated.

Consent for publication
Written consent for publication obtained from all participants.

Competing interests
The authors report no conflict of interest.

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