# NANOSILIMAGNA PROJECT

**Report of a Project performed for**

## Spiegel Online

**by the**


## Osteoporosis Research Center
## Creighton University
**Omaha, Nebraska**

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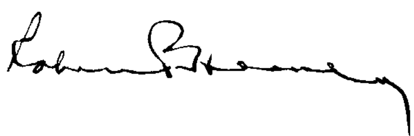
### *Authentication*

This is to certify that the undersigned performed the studies described in this report and that the results contained herein accurately represent what was found.



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## *Introduction and Background*

Neosino Nanotechnologies Deutschland Vertriebs-GmbH, Griesheim, Germany, markets in Germany a nutritional supplement containing calcium, silicon, and magnesium (Nanosilimagna), in which the elements concerned are said to be in the form of nano particles, and for which the manufacturer claims absorbability superior to that of other physical forms of the same elemental nutrients.

The news website Spiegel Online challenged the claims by the manufacturer of Nanosilimagna. Subsequently Spiegel Online asked the Osteoporosis Research Center (ORC) of Creighton University, Omaha, Nebraska, USA, to address the question of absorption of calcium and magnesium from Nanosilimagna with a specific study in 12 volunteers. As a comparator supplement, a widely used and inexpensive effervescent tablet from a German supermarket was used. Creighton's ORC has extensive experience in measuring absorbability of various calcium sources, both food and supplement (see list of publications in Appendix II).

This report describes the study performed by Creighton's ORC and presents its findings.

### *Materials and Methods*

Participants. Fourteen healthy adult men and women were recruited. Target enrollment was 12, and the project was over-recruited to ensure completion of 12 subjects. Participants had to have been in good general health, to have BMI values between 19 and 31 kg/m$^2$ and not to have disorders or be receiving medications that might influence absorption. Pertinent personal data are set forth in Table 1. The study was approved by the Creighton University Institutional Review Board, and each participant gave written consent (see Appendix I). One subject (NSM-12) dropped from study prior to the first test visit. He was replaced by the planned over-recruitment. Another subject (NSM-06) was not able to produce a urine sample at the two hour time point, but did so at the 6-hour time point. Hence we have data for the total excretion for him, but not for the earlier/later segments of that 6-hour period.

Protocol. Each participant was studied three times, once on each of the two test products, and once on no calcium or magnesium (blank). In all cases except one, the three tests were carried out on a M-W-F schedule within a single week. The mid-week test provided the "blank", or no supplement, test and at the Monday and Friday sessions one or other of the two test sources was given in a random sequence.

The primary outcome measures were the rises in urine calcium and magnesium from zero to two hours and from two to six hours (and the sum of the two, i.e., zero to six hours), for each of the supplement sources, relative to the excretion on the blank (or no-supplement) day. Secondary measures included excretion of creatinine and sodium in the same urine collections. The former was to permit adjustment for possibly incomplete collections, and the latter to signal possible alterations in urinary calcium excretion caused, not so much by the test sources, but by large variations in salt intake or salt loss on the day(s) preceding any given test.

The test sources were given at the mid point of a test breakfast after a 10 hour overnight fast, and with 200 mL deionized water. The breakfast consisted of three pieces of low calcium bread, baked in the ORC kitchen, buttered, plus a cup of coffee, tea, or water (with artificial sweetener, if desired). In addition to the minerals provided by the test sources, the test breakfast was estimated to contain 4.2 mg calcium and 6.0 mg magnesium.

Each participant emptied his or her bladder immediately before the test breakfast, and then collected all urine in two pools (zero to two hours and two to six hours). After breakfast, each participant was given at least 470 mL deionized water to drink over the course of the morning. No other food or drink was permitted until completion of the six hour collection. At completion of each test day, participants were given a meal voucher for lunch in the Medical Center cafeteria.

Prior to beginning the series of three tests, each participant received instructions from the ORC dietitian designed to limit individual calcium and sodium intakes and to maintain constancy of those intakes for seven days prior to beginning the test series, as well as during the test week. (Thus, rather than imposing an artificial restriction for either sodium or calcium, the attempt was made to stabilize the individual's customary intake of the two nutrients prior to and during the test week.)

Although sodium was measured on the specimens produced in this study, there was no significant relationship found between sodium and calcium excretion, and hence no attempt was made to adjust measured calcium excretion for the sodium content of the same specimens.

Test Sources. Nanosilimagna was dosed as five gelatin capsules of the marketed product, the blank as five empty gelatin capsules, and the effervescent calcium and magnesium sources as one tablet of *Ja! Calcium* and *Ja! Magnesium* (Rewe-Handelsgruppe GmbH, Köln), each dissolved in 100 mL of deionized water (plus three small deionized water rinses to ensure quantitative ingestion). The Nanosilimagna and blank were each swallowed with 200 ml of deionized water, so as to equalize fluid intake across all three sources. The Nanosilimagna product provided, as labeled, 435 mg calcium and 266.5 mg magnesium, the blank none of either nutrient, and the effervescent tablet, 500 mg calcium and 200 mg magnesium.

Statistical Analysis. Analysis was by ANOVA, comparing source and order, using SPSS for Windows (Version 13.0), as well as the statistical routines in Microsoft Excel (2003). The primary variable for each nutrient was the within-subject difference in urinary excretion of calcium and magnesium between a given test source and the blank (i.e., the rise reflecting absorptive calcemia and magnesemia). The primary comparison was between the within-subject difference in excretion for Nanosilimagna and for the effervescent tablet sources. The null hypothesis tested was that the rise on Nanosilimagna was not different from the corresponding rise for the effervescent sources.

Because of differences in the calcium and magnesium load sizes for the two products as marketed, it was necessary to adjust measured excretions to eliminate any difference in excretion due to variation in load. For calcium, this was done in two ways: 1) straightforward linear proportion (i.e., effervescent load/nanosilimagna load); and 2) a linear proportion reduced by the predicted difference in absorption fraction due to a difference in load of any specified size (64.5 mg in this case).[1] Using the linear approach, measured calcium excretion following Nanosilimagna was increased by a factor of 1.149, and with the non-linear method, by 1.116. As no data are available for the relationship of load to magnesium absorption, only the linear adjustment (a factor of 0.752) was used for that element.

[1] Heaney RP, Weaver CM, Fitzsimmons ML. The influence of calcium load on absorption fraction. J Bone Miner Res 11(5):1135-1138, 1990.

## *Results*

Table 2 sets forth the four relevant excretion variables for the basal, or no-load state, in each of the 13 individuals completing the study. These are the absolute excretions of calcium and magnesium and the corresponding values normalized per g creatinine, as well as the sodium content of the urine specimens (in mEq). These data, in effect, characterize the mineral excretory pattern of the participants. In brief none stands out as unusual.

Table 3 sets forth the principal outcome variables of this study design, i.e., the within-subject difference between the no-load and the test load conditions, using the non-linear adjustment described above to compensate for differences in calcium load size. In brief, the excretion of calcium, both early and late, was higher for the effervescent tablets than for the Nanosilimagna, though the difference was not statistically significant. Using instead the linear adjustment for calcium for nanosilimagna produced calcium excretion values about 3% higher than shown in Table 3. They still were marginally lower than the values obtained with the effervescent tablets and, as with the non-linear adjustment, not statistically significantly different.

When the values are expressed per g creatinine, the calcium excretory pattern and conclusions are not altered.

The magnesium excretion, however, was significantly lower for Nanosilimagna both early and late, and, in fact, the Nanosilimagna magnesium excretion values were not significantly different from zero.

Table 4 and the figure below set forth the within-subject differences between the two test sources, showing exactly what has just been described, i.e., no significant difference between sources for calcium and significantly lower excretion of magnesium for Nanosilimagna.

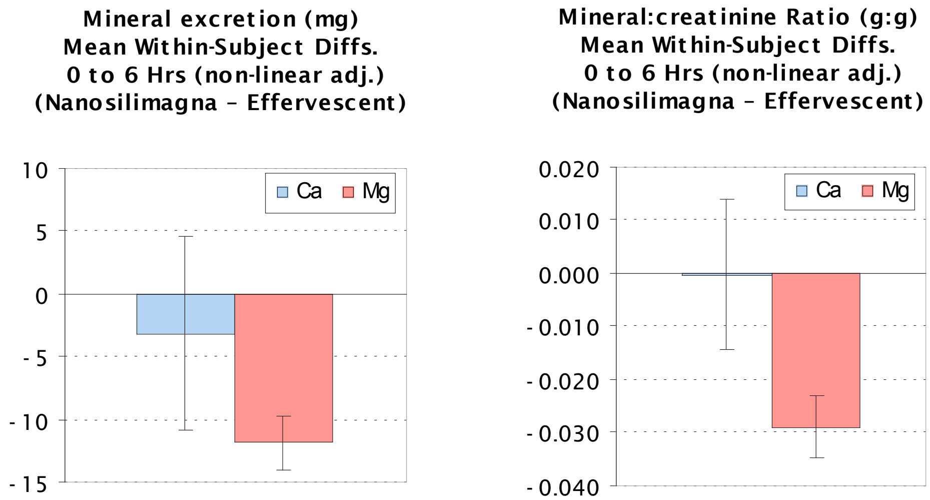


Figure. Mean within-subject differences (± 1 SEM) between urinary excretion of calcium and magnesium for Nanosilimagna and effervescent tablets. The left hand panel is for the differences in total measured excretion, and the right hand panel for the excretion values adjusted for the creatinine content of the same specimens. Negative values favor the effervescent tablet source.

### *Interpretation/Comment*

Extracellular fluid calcium ion concentration is tightly regulated, normally varying by no more than 1 or 2%. However, when calcium is absorbed from the upper intestine, it typically enters the blood stream more rapidly than body systems can fully compensate. As a result, blood calcium tends to rise during the absorptive process. The rise for calcium is small (on the order of only 1% for each 100 mg calcium ingested); nevertheless some of that excess is spilled into the urine, which is why urinary excretion has often been used as an indirect measure of absorption. It must be noted, however, that it is a relatively weak measure, as many other factors besides absorption influence urinary calcium excretion. We attempted to compensate for these sources of variation by prescribing a constant intake of protein and salt (the two principal dietary factors affecting urine calcium) over the course of the three study days and for a 5–7 day pre-study period of equilibration on the constant intake. Also, the use of a crossover design, as in this study, largely eliminates inter-individual sources of variability.

The data generated in this study provide no evidence either for greater absorption or for faster absorption of the calcium in Nanosilimagna than from the effervescent calcium sources. By contrast, Nanosilimagna was clearly inferior to the effervescent tablet in magnesium absorption. At no time point did the magnesium excretion differ significantly from zero, a finding compatible with a conclusion of essentially zero bioavailability for the magnesium component of Nanosilimagna.

Because of the imprecision inherent in the urine excretion method, as well as the small sample size, it is not possible to exclude some small difference in absorbability of calcium from the two sources (in one direction or the other). Nevertheless there is no hint of significant superiority of Nanosilimagna in the data generated by this study.

**Table 1. Personal data**

| ID# | DOB | Age | Sex | Study Date | Smoke | Ht (m) | Wt (kg) | Seq* |
|---|---|---|---|---|---|---|---|---|
| NSM-01 | 12/15/1951 | 54.5 | m | 6/5/06 | former | 1.897 | 102.54 | ne |
| NSM-02 | 4/14/1952 | 54.2 | m | 6/5/06 | never | 1.760 | 76.45 | en |
| NSM-03 | 10/10/1963 | 42.7 | f | 6/5/06 | former | 1.734 | 84.39 | ne |
| NSM-04 | 1/26/1971 | 35.4 | f | 6/5/06 | never | 1.708 | 78.04 | ne |
| NSM-05 | 1/17/1962 | 44.4 | f | 6/5/06 | former | 1.683 | 80.76 | ne |
| NSM-06 | 8/31/1955 | 50.8 | m | 6/5/06 | current | 1.730 | 80.31 | ne |
| NSM-07 | 6/26/1976 | 30.0 | m | 6/5/06 | never | 1.801 | 83.03 | ne |
| NSM-08 | 4/5/1966 | 40.2 | f | 6/5/06 | never | 1.696 | 71.23 | ne |
| NSM-09 | 4/24/1985 | 21.1 | f | 6/5/06 | never | 1.711 | 59.89 | en |
| NSM-10 | 4/28/1984 | 22.1 | m | 6/5/06 | never | 1.817 | 82.12 | en |
| NSM-11 | 8/26/1985 | 20.8 | m | 6/5/06 | never | 1.814 | 74.41 | en |
| NSM-12 | | | | | | | | en |
| NSM-13 | 1/8/1985 | 21.4 | m | 6/19/06 | never | 1.845 | 74.18 | en |
| NSM-14 | 9/4/1962 | 43.8 | f | 6/19/06 | former | 1.687 | 74.64 | en |
| | | | | | | | | |
| **Mean** | | 37.0 | | | | 1.760 | 78.62 | |
| **StDev** | | 12.79 | | | | 0.068 | 9.61 | |
| **N** | | 13 | | | | 13 | 13 | |
| **SEM** | | 3.55 | | | | 0.019 | 2.67 | |

* n = nanosilimagna; e = effervescent tablets

**Table 2. Baseline Excretion values (no mineral load)**

| | *0–2 hr collection* | | | | | | *2–6 hr collection* | | | | | | *0–6 hr collection* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID | crt (mg) | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) | Na (mEq) | crt (mg) | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) | Na (mEq) | crt (mg) | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) | Na (mEq) |
| NSM-01 | 85.8 | 4.29 | 3.30 | 0.0500 | 0.0385 | 8.9 | 254.6 | 7.37 | 7.37 | 0.0289 | 0.0289 | 24.1 | 340.4 | 11.66 | 10.67 | 0.0343 | 0.0313 | 33.0 |
| NSM-02 | 146.8 | 20.76 | 10.02 | 0.1415 | 0.0683 | 14.7 | 302.7 | 40.90 | 21.27 | 0.1351 | 0.0703 | 45.8 | 449.4 | 61.66 | 31.29 | 0.1372 | 0.0696 | 60.5 |
| NSM-03 | 132.3 | 27.59 | 10.96 | 0.2086 | 0.0829 | 17.4 | 217.1 | 20.40 | 11.84 | 0.0939 | 0.0545 | 42.1 | 349.4 | 47.99 | 22.81 | 0.1373 | 0.0653 | 59.5 |
| NSM-04 | 119.6 | 5.46 | 8.58 | 0.0457 | 0.0717 | 16.1 | 232.5 | 3.75 | 9.75 | 0.0161 | 0.0419 | 22.5 | 352.1 | 9.21 | 18.33 | 0.0262 | 0.0521 | 38.6 |
| NSM-05 | 110.2 | 18.04 | 3.01 | 0.1636 | 0.0273 | 14.0 | 210.9 | 9.69 | 3.99 | 0.0459 | 0.0189 | 15.4 | 321.1 | 27.73 | 7.00 | 0.0863 | 0.0218 | 29.4 |
| NSM-06 | 125.6 | 20.81 | 13.07 | 0.1658 | 0.1041 | 12.4 | 323.7 | 37.85 | 27.39 | 0.1169 | 0.0846 | 30.9 | 449.3 | 58.66 | 40.46 | 0.1306 | 0.0901 | 43.3 |
| NSM-07 | 224.0 | 12.60 | 4.90 | 0.0563 | 0.0219 | * | 380.0 | 28.88 | 6.84 | 0.0760 | 0.0180 | * | 604.0 | 41.48 | 11.74 | 0.0687 | 0.0194 | * |
| NSM-08 | 93.5 | 13.50 | 9.41 | 0.1443 | 0.1006 | 6.9 | 181.3 | 11.29 | 11.97 | 0.0623 | 0.0660 | 21.9 | 274.8 | 24.78 | 21.38 | 0.0902 | 0.0778 | 28.8 |
| NSM-09 | 112.7 | 18.08 | 6.55 | 0.1605 | 0.0581 | 8.4 | 197.4 | 23.27 | 9.87 | 0.1179 | 0.0500 | 21.2 | 310.1 | 41.34 | 16.42 | 0.1333 | 0.0530 | 29.5 |
| NSM-10 | 169.0 | 30.72 | 14.78 | 0.1818 | 0.0875 | 19.2 | 323.9 | 13.43 | 9.48 | 0.0415 | 0.0293 | 32.4 | 492.9 | 44.15 | 24.26 | 0.0896 | 0.0492 | 51.6 |
| NSM-11 | 204.6 | 15.28 | 10.62 | 0.0747 | 0.0519 | 22.3 | 319.5 | 11.58 | 11.58 | 0.0362 | 0.0362 | 14.8 | 524.1 | 26.86 | 22.19 | 0.0512 | 0.0423 | 37.1 |
| NSM-12 | | | | | | | | | | | | | | | | | | |
| NSM-13 | 139.2 | 8.72 | 7.89 | 0.0627 | 0.0567 | 1.2 | 290.4 | 14.39 | 13.07 | 0.0495 | 0.0450 | 4.8 | 429.6 | 23.11 | 20.96 | 0.0538 | 0.0488 | 6.0 |
| NSM-14 | 121.7 | 15.55 | 7.77 | 0.1278 | 0.0639 | 16.2 | 220.2 | 6.88 | 11.70 | 0.0313 | 0.0531 | 28.2 | 341.8 | 22.43 | 19.47 | 0.0656 | 0.0570 | 44.4 |
| Mean | 137.30 | 16.26 | 8.53 | 0.1218 | 0.0641 | 13.1 | 265.7 | 17.666 | 12.009 | 0.0655 | 0.0459 | 25.3 | 403.0 | 33.928 | 20.537 | 0.0849 | 0.0521 | 38.5 |
| StDev | 40.59 | 7.77 | 3.52 | 0.0564 | 0.0257 | 5.9 | 61.3 | 11.895 | 6.140 | 0.0389 | 0.0200 | 11.5 | 97.1 | 16.642 | 8.739 | 0.0395 | 0.0207 | 15.1 |
| N | 13 | 13 | 13 | 13 | 13 | 12 | 13 | 13 | 13 | 13 | 13 | 12 | 13 | 13 | 13 | 13 | 13 | 12 |
| SEM | 11.26 | 2.15 | 0.98 | 0.016 | 0.007 | 1.70 | 17.01 | 3.299 | 1.703 | 0.0108 | 0.0056 | 3.33 | 26.92 | 4.616 | 2.424 | 0.0110 | 0.0057 | 4.35 |

* outofrange (low)

## Table 3a. Nanosilimagna Excretion Values (differences from no load)

| | *0– 2 hr collection* | | | | *2–6 hr collection* | | | | *0–6 hr collection* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) |
| NSM-01 | 11.46 | 2.01 | 0.0434 | -0.0070 | 24.99 | 6.26 | 0.0855 | 0.0193 | 36.45 | 8.26 | 0.0723 | 0.0106 |
| NSM-02 | 10.80 | 3.81 | 0.0546 | 0.0176 | 8.91 | 1.11 | 0.0255 | 0.0019 | 19.72 | 4.92 | 0.0356 | 0.0073 |
| NSM-03 | -7.24 | -4.65 | -0.0434 | -0.0316 | 21.66 | 3.38 | 0.0782 | 0.0078 | 14.42 | -1.27 | 0.0325 | -0.0067 |
| NSM-04 | 16.71 | 0.34 | 0.0799 | -0.0212 | 36.09 | 6.04 | 0.1645 | 0.0297 | 52.80 | 6.38 | 0.1300 | 0.0102 |
| NSM-05 | -3.32 | -0.40 | 0.0130 | 0.0041 | 81.98 | 11.74 | 0.3125 | 0.0426 | 78.66 | 11.35 | 0.2274 | 0.0323 |
| NSM-06 | | | | | | | | | 43.08 | 4.45 | 0.0805 | 0.0031 |
| NSM-07 | -0.88 | -2.70 | 0.0883 | 0.0052 | -1.41 | -3.03 | 0.0699 | 0.0022 | -2.29 | 1.57 | 0.0645 | 0.0099 |
| NSM-08 | 10.07 | -0.72 | 0.0342 | -0.0348 | 27.30 | 2.80 | 0.1381 | 0.0107 | 37.38 | 2.08 | 0.1013 | -0.0055 |
| NSM-09 | 0.35 | -0.42 | 0.0059 | -0.0028 | 31.69 | 6.71 | 0.1590 | 0.0335 | 32.03 | 6.29 | 0.1040 | 0.0205 |
| NSM-10 | -5.40 | -6.22 | -0.0366 | -0.0384 | 31.53 | 2.82 | 0.0960 | 0.0083 | 26.13 | -3.40 | 0.0506 | -0.0076 |
| NSM-11 | 20.43 | -0.99 | 0.0443 | -0.0198 | 28.74 | 3.35 | 0.0832 | 0.0080 | 49.16 | 2.35 | 0.0680 | -0.0038 |
| NSM-12 | | | | | | | | | | | | |
| NSM-13 | 2.34 | -2.39 | 0.0078 | -0.0216 | 7.46 | -2.36 | 0.0260 | -0.0080 | 9.80 | -4.75 | 0.0200 | -0.0125 |
| NSM-14 | -10.05 | -1.56 | -0.0851 | -0.0157 | 1.09 | -2.03 | 0.0109 | -0.0021 | -8.96 | -3.59 | -0.0233 | -0.0071 |
| Mean | 3.77 | -1.16 | 0.0172 | -0.0138 | 25.00 | 3.06 | 0.1041 | 0.0128 | 29.88 | 2.66 | 0.0741 | 0.0039 |
| StDev | 9.89 | 2.70 | 0.0517 | 0.0175 | 22.00 | 4.31 | 0.0825 | 0.0154 | 23.90 | 4.92 | 0.0610 | 0.0129 |
| N | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 13 | 13 | 13 | 13 |
| SEM | 2.85 | 0.78 | 0.0149 | 0.0050 | 6.35 | 1.24 | 0.0238 | 0.0044 | 6.63 | 1.36 | 0.0169 | 0.0036 |

## Table 3b. Effervescent Tablets Excretion Values (differences from no load)

| | *0– 2 hr collection* | | | | *2–6 hr collection* | | | | *0–6 hr collection* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ID | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) |
| NSM-01 | 5.27 | 3.87 | 0.0056 | 0.0032 | 21.99 | 13.46 | 0.0596 | 0.0339 | 27.26 | 17.33 | 0.0430 | 0.0243 |
| NSM-02 | 23.21 | 6.73 | 0.0685 | 0.0117 | 13.18 | -3.42 | 0.0556 | -0.0073 | 36.39 | 3.31 | 0.0617 | 0.0006 |
| NSM-03 | -13.57 | 2.06 | -0.1086 | 0.0100 | 25.36 | 25.34 | 0.1000 | 0.1030 | 11.80 | 27.40 | 0.0216 | 0.0682 |
| NSM-04 | 5.57 | 7.00 | 0.0362 | 0.0438 | 16.82 | 15.06 | 0.0758 | 0.0689 | 22.39 | 22.05 | 0.0619 | 0.0605 |
| NSM-05 | 8.72 | 5.57 | 0.0530 | 0.0422 | 46.61 | 13.49 | 0.2266 | 0.0657 | 55.32 | 19.06 | 0.1653 | 0.0572 |
| NSM-06 | -13.67 | -3.67 | -0.1170 | -0.0399 | 43.47 | 24.29 | 0.1157 | 0.0632 | 29.80 | 20.62 | 0.0478 | 0.0331 |
| NSM-07 | 49.11 | 10.81 | 0.1938 | 0.0418 | 18.08 | 5.46 | 0.0688 | 0.0199 | 67.19 | 16.27 | 0.1216 | 0.0296 |
| NSM-08 | 10.84 | 8.17 | -0.0058 | -0.0006 | 12.58 | 6.56 | 0.1066 | 0.0651 | 23.42 | 14.72 | 0.0618 | 0.0361 |
| NSM-09 | 6.38 | 3.50 | 0.0607 | 0.0328 | 39.83 | 14.51 | 0.2121 | 0.0775 | 46.21 | 18.01 | 0.1568 | 0.0611 |
| NSM-10 | -4.10 | -6.26 | -0.0348 | -0.0404 | 25.45 | 2.40 | 0.0710 | 0.0051 | 21.36 | -3.86 | 0.0348 | -0.0105 |
| NSM-11 | 0.23 | -0.75 | 0.0122 | 0.0034 | 32.36 | 18.01 | 0.0826 | 0.0438 | 32.59 | 17.26 | 0.0572 | 0.0296 |
| NSM-12 | | | | | | | | | | | | |
| NSM-13 | 2.36 | -1.05 | 0.0091 | -0.0123 | 20.76 | 8.59 | 0.0712 | 0.0294 | 23.11 | 7.54 | 0.0500 | 0.0152 |
| NSM-14 | 2.14 | 2.05 | 0.0046 | 0.0096 | 30.23 | 7.31 | 0.1264 | 0.0276 | 32.36 | 9.36 | 0.0829 | 0.0212 |
| Mean | 6.34 | 2.92 | 0.0137 | 0.0081 | 26.67 | 11.62 | 0.1055 | 0.0458 | 33.01 | 14.54 | 0.0743 | 0.0328 |
| StDev | 16.12 | 4.90 | 0.0789 | 0.0280 | 11.22 | 8.26 | 0.0550 | 0.0313 | 15.23 | 8.42 | 0.0454 | 0.0239 |
| N | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 | 13 |
| SEM | 4.47 | 1.36 | 0.0219 | 0.0078 | 3.11 | 2.29 | 0.0153 | 0.0087 | 4.22 | 2.33 | 0.0126 | 0.0066 |

| Table 4. Within-Subject Differences (Nanosilimagna – Effervescent Tablets) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***0– 2 hr collection*** | | | | ***2–6 hr collection*** | | | | ***0–6 hr collection*** | | | |
| ID | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) | Ca (mg) | Mg (mg) | Ca/crt (g/g) | Mg/crt (g/g) |
| NSM-01 | 6.187 | -1.865 | 0.0379 | -0.0102 | 3.003 | -7.206 | 0.0259 | -0.0147 | 9.190 | -9.071 | 0.0293 | -0.0137 |
| NSM-02 | -12.406 | -2.917 | -0.0139 | 0.0059 | -4.263 | 4.526 | -0.0301 | 0.0092 | -16.669 | 1.609 | -0.0261 | 0.0067 |
| NSM-03 | 6.325 | -6.710 | 0.0652 | -0.0416 | -3.701 | -21.960 | -0.0218 | -0.0953 | 2.624 | -28.671 | 0.0109 | -0.0748 |
| NSM-04 | 11.149 | -6.652 | 0.0437 | -0.0651 | 19.266 | -9.016 | 0.0888 | -0.0392 | 30.416 | -15.667 | 0.0680 | -0.0504 |
| NSM-05 | -12.040 | -5.966 | -0.0400 | -0.0381 | 35.372 | -1.746 | 0.0859 | -0.0231 | 23.332 | -7.712 | 0.0622 | -0.0248 |
| NSM-06 | | | | | | | | | 13.280 | -16.167 | 0.0327 | -0.0300 |
| NSM-07 | -49.986 | -13.508 | -0.1055 | -0.0365 | -19.488 | -8.487 | 0.0011 | -0.0177 | -69.474 | -14.698 | -0.0571 | -0.0197 |
| NSM-08 | -0.769 | -8.892 | 0.0401 | -0.0342 | 14.727 | -3.752 | 0.0315 | -0.0544 | 13.958 | -12.643 | 0.0395 | -0.0416 |
| NSM-09 | -6.031 | -3.921 | -0.0549 | -0.0356 | -8.145 | -7.799 | -0.0531 | -0.0440 | -14.176 | -11.720 | -0.0529 | -0.0407 |
| NSM-10 | -1.301 | 0.045 | -0.0019 | 0.0020 | 6.078 | 0.415 | 0.0250 | 0.0032 | 4.778 | 0.461 | 0.0158 | 0.0029 |
| NSM-11 | 20.198 | -0.246 | 0.0322 | -0.0232 | -3.622 | -14.655 | 0.0006 | -0.0358 | 16.575 | -14.901 | 0.0108 | -0.0334 |
| NSM-12 | | | | | | | | | | | | |
| NSM-13 | -0.018 | -1.343 | -0.0013 | -0.0093 | -13.296 | -10.948 | -0.0452 | -0.0374 | -13.314 | -12.291 | -0.0300 | -0.0277 |
| NSM-14 | -12.184 | -3.611 | -0.0897 | -0.0254 | -29.138 | -9.342 | -0.1156 | -0.0297 | -41.322 | -12.953 | -0.1062 | -0.0283 |
| Mean | -4.240 | -4.632 | -0.0073 | -0.0259 | -0.267 | -7.498 | -0.0006 | -0.0316 | -3.139 | -11.879 | -0.0002 | -0.0289 |
| StDev | 17.528 | 3.945 | 0.0553 | 0.0203 | 17.584 | 6.964 | 0.0581 | 0.0274 | 27.826 | 7.600 | 0.0511 | 0.0214 |
| N | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 12 | 13 | 13 | 13 | 13 |
| SEM | 5.060 | 1.139 | 0.0159 | 0.0059 | 5.076 | 2.010 | 0.0168 | 0.0079 | 7.718 | 2.108 | 0.0142 | 0.0059 |
| t | -0.8379 | -4.0676 | -0.4607 | -4.4272 | -0.0527 | -3.7295 | -0.0346 | -3.9868 | -0.4067 | -5.6354 | -0.0171 | -4.8626 |
| P | NS | <0.01 | NS | <0.01 | NS | <0.01 | NS | <0.01 | NS | <0.01 | NS | <0.01 |

# Appendix I

## Consent Form

## CONSENT FORM

**Title of Study**: Comparative Absorbability of Two Calcium Sources

**Principal Investigator**: Robert P. Heaney, M.D.
**Co-Investigators:** M Susan Dowell, RN, PhD
Karen Rafferty, R.D.
Creighton University
Osteoporosis Research Center
601 North 30th Street; Suite 5766
Omaha, NE 68131
Phone: (402)-280-4029

**Purpose**: The purpose of this research study is to compare the absorbability of a newly developed calcium–magnesium supplement (produced and promoted in Germany) with an established, effervescent calcium source in longstanding international distribution. There will be 12–14 healthy males and females participating in this study.

**Procedure**: You will come to the research unit three times in one week. One week prior to the study week and during the study week you will consume a low calcium, constant sodium diet.

Day #1 of the study week: You will arrive fasting and will then be served the standardized test breakfast which consists of 2–3 pieces of white, buttered toast and a cup of coffee/tea/water. Mid-way through the breakfast you will swallow the calcium supplement products with a glass of water. Following breakfast, you will have nothing further to eat for the next six hours except water. During this same 6-hour period you will collect urine in a container that we provide. You will return the urine sample to the research unit at the end of the 6-hour collection period.

Day #2 of the study week: You will maintain the low calcium, constant sodium diet.
Day #3 of the study week: repeat of Day #1
Day #4 of the study week: repeat of Day #2
Day #5 of the study week: repeat of Day #1

**Potential Risks**: None.

**Compensation**: You will also receive a stipend of $30.00 for each visit or a total of $150.00 on completion of the project.

**Benefits**: By participating in this research project, you will help us to learn more 1) about the absorption of calcium, an essential nutrient, and 2) about factors that may make some sources of calcium better than others.

**Confidentiality**:  We will not release any information about you without your permission, except as required by law.  Please note, however, that representatives of the Creighton University Institutional Review Board and the U.S. Food & Drug Administration may legally review research records which may identify you by name.  Results from this study will, when presented orally or published in the medical literature, be disclosed in a way that does not reveal your identify.
If you have any questions about this project, you may call one of the investigators listed above.

**Research-related Injury**:  The investigators will make every effort to prevent study-related injuries and illnesses.  If you are injured or become ill while you are in the study and the illness or injury is due to your participation in this study, you will receive necessary medical care at the usual charge.  The costs of this care will be charged to you or your health-insurer.  No funds are available from Creighton University to repay you or compensate you for a study-related injury or illness.  There is also no compensation for payment of your lost wages or other losses.

By signing this consent form you will not be waiving any of your legal rights which you otherwise would have as a subject in a research study.

**CONSENT STATEMENT**
I understand that I am free to refuse to participate in this research project or to withdraw my consent and discontinue participation in the project at any time without prejudice to me or effect on my medical care.  All my questions regarding this project have been answered.  I agree to participate in the project as described above.

| | |
|---|---|
| ______________________________ | ______________________________ |
| Signature of Subject | Signature of Witness |
| ______________________ | ______________________ |
| Date Signed | Date Signed |

If you are not satisfied with the manner in which this study is being conducted, you may report (anonymously if you so choose) any complaints to the Institutional Review Board by calling (402)-280-2126, or addressing a letter to the Institutional Review Board, Office of Grants Administration,  Creighton University, 2500 California Plaza, Omaha, NE 68178.

A COPY OF THIS FORM HAS BEEN GIVEN TO ME.  _______________
Subject's Initials

I have discussed with this subject (and, if required, the subject's guardian) the procedure(s) described above and the risks involved; I believe s/he understands the contents of the consent form, and is competent to give legally effective and informed consent.

| | |
|---|---|
| _______________________ | ___________________ |
| Signature of Investigator | Date Signed |

# Appendix II

# Papers on Calcium Absorption Measurement From Creighton University's Osteoporosis Research Center

## Methodologic Papers

Heaney RP, Recker RR. Estimation of true calcium absorption. *Ann Int Med* 103:516-521,1985.

Heaney RP, Recker RR. Estimating true fractional calcium absorption. *Ann Int Med* 108:905-906, 1988.

Heaney RP, Dowell MS, Wolf RL. Estimation of true calcium absorption in men. *Clin Chem* 48(5):786-788, 2002.

Heaney RP, Dowell MS, Barger-Lux MJ. Absorption of calcium as the carbonate and citrate salts, with some observations on method. *Osteoporos Int* 9:19-23, 1999.

Heaney RP, Dowell MS, Rafferty K, Bierman J. Bioavailability of the calcium in fortified soy imitation milk, with some observations on method. *Am J Clin Nutr* 71:1166-1169, 2000.

## Applications – Peer Reviewed Publications

Heaney RP, Recker RR. Distribution of calcium absorption in middle-aged women. *Am J Clin Nutr* 43:299-305, 1986.

Smith KT, Heaney RP, Flora L, Hinders S. Calcium absorption from a new calcium delivery system (CCM). *Calcif Tissue Int 42:35 1-352, 1987.*

Heaney RP, Recker RR, Hinders SM. Variability of calcium absorption. *Am J Clin Nutr* 47:262-264, 1988.

Recker RR, Bammi A, Barger-Lux MJ, Heaney RP. Calcium absorbability from milk products, an imitation milk, and calcium carbonate. *Am J Clin Nutr* 47:93-95, 1988.

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Heaney RP, Smith KT, Recker RR, Hinders SM. Meal effects on calcium absorption. *Am J C/in Nutr* 49:372-376, 1989.

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Barger-Lux MJ, Heaney RP, Recker RR. Time course of calcium absorption in humans: evidence for a colonic component. *Calcif Tissue Int* 44:308-311, 1989.

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Heaney RP, Weaver CM, Fitzsimmons ML. The influence of calcium load on absorption fraction. *J Bone Miner Res* 11(5):1135-1138, 1990.

Heaney RP, Weaver CM, Fitzsimmons ML, Recker RR. Calcium absorptive consistency. *J Bone Miner Res* 11(5):1139-1142, 1990.

Weaver CM, Heaney RP. Isotopic exchange of ingested calcium between labeled sources. Does dietary calcium form a common absorptive pool? *Calcif Tissue Int* 49:244-247,1991.

Heaney RP, Weaver CM, Fitzsimmons ML. Soybean phytate content: effect on calcium absorption. *Am J C/in Nutr* 53:741-744, 1991.

Weaver CM, Heaney RP, Martin BR, Fitzsimmons ML. Human calcium absorption from whole wheat products. *J Nutr* 121:1769-1775, 1991.

Weaver CM, Heaney RP, Martin BR, Fitzsimmons ML. Extrinsic vs. intrinsic labeling of the calcium in whole wheat flour. *Am J Clin Nutr* 55:452-454, 1992.

Heaney RP, Weaver CM, Hinders SM, Martin B, Packard P. Absorbability of calcium from *Brassica* vegetables. *J Food Sci* 58:1378-1380, 1993.

Weaver CM, Heaney RP, Proulx WR, Hinders SM, Packard PT. Absorbability of calcium from common beans. *J Food Sci* 58:1401-1403,1993.

Heaney RP, Dowell MS. Absorbability of calcium in a high calcium mineral water. *Osteoporos Int* 4:323-324,1994.

Abrams SA, Yergey AL, Heaney RP. Relationship between balance and dual tracer isotopic measurements of calcium absorption and excretion. *J Clin Endocrinol Metab* 79:965-969,1994.

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Weaver CM, Heaney RP, Teegarden D, Hinders SM. Wheat bran abolishes the inverse relationship between calcium load size and absorption fraction in women. *J Nutr* 126:303-307,1996.

Weaver CM, Heaney RP, Nickel KP, Packard PT. Calcium bioavailability from high oxalate vegetables: Chinese vegetables, sweet potatoes, and rhubarb. *J Food Sci* 62:525-525, 1997.

Hanes DA, Weaver CM, Heaney RP, Wastney M. Absorption of calcium oxalate does not require dissociation in rats. *J Nutr* 129:170-173, 1999.

Heaney RP, Dowell MS, Barger-Lux MJ. Absorption of calcium as the carbonate and citrate salts, with some observations on method. *Osteoporos Int* 9:19-23, 1999.

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Ensrud KE, Duong T, Cauley JA, Heaney RP, Wolf RL, Harris E, Cummings SR. Low fractional calcium absorption increases the risk of hip fracture in women with low calcium intake. *Ann Intern Med* 132:345-353, 2000.

Weaver CM, Heaney RP, Connor L, Martin BR, Smith DL, Nielsen S. Bioavailability of calcium from tofu as compared with milk in premenopausal women. *J Food Sci* 67(8):3144-3147, 2002.

Martin BR, Weaver CM, Heaney RP, Packard PT, Smith DL. Calcium absorption from three salts and $CaSO_4$-fortified bread in premenopausal women. *J Agric Food Chem* 50:3874-3876, 2002.

Griffin IJ, Hicks PMD, Heaney RP, Abrams SA. Enriched chicory inulin increases calcium absorption mainly in adolescents with lower calcium absorption. *Eur J Clin Nutr* (submitted) 2002.

Heaney RP. Quantifying human calcium absorption using pharmacokinetic methods. *J Nutr* 133:1224-1226, 2003.

O'Connell MB, Madden D, Murray A, Heaney RP, Kerzner LJ. Effect of proton pump inhibition on $^{45}$Calcium carbonate absorption from an empty stomach: a randomized, double-blind, placebo-controlled, crossover clinical trial. *Am J Med* 118:778-781, 2005.

Barger-Lux MJ, Heaney RP. Calcium absorptive efficiency is positively related to body size. *J Clin Endocrinol Metab* 90:5118-5120, 2005.

Heaney RP. Absorbability and utility of calcium in mineral water. *Am J Clin Nutr* (in press) 2006.