STATISTICAL ANALYSIS PLAN

Official title: Evaluation of the effects of taking bicarbonate-calcium water in premenopausal and postmenopausal women as prevention of osteoporosis

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Data from scientific literature were used to calculate the sample size and evaluate the power of the clinical study, and in particular: • Meunier, PJ, Jenvrin, C., Munoz, F., de la Gueronniere, V., Garnero, P., & Menz, M. (2005).
Consumption of a high calcium mineral water lowers biochemical indices of bone remodeling in postmenopausal women with low calcium intake. *Osteoporosis international,* 16(10), 1203-1209

• Wynn, E., Krieg, M. A., Aeschlimann, J. M., & Burckhardt, P. (2009). Alkaline mineral water lowers bone resorption even in calcium sufficiency:: Alkaline mineral water and bone metabolism. *Bone,* 44(1), 120-124

As a reference for the statistical calculations, a biochemical parameter was considered, which represents a primary endpoint of the study, the value of CTX (C-terminal telopeptide of type I collagen), a serum marker of bone turnover.

Both of the studies cited analyzed the effects of calcium-rich bicarbonate alkaline water in samples of women: in the first case in menopausal women and in the second in women in the 18-45 age range.

The results of the two studies are reported in the summary table.

Meunier et al., 2005		
S. CTX (pmol/L)	Control group (n=43) [2]	Intervention group (n=44)
baseline	0.503±0.039	0.542±0.032
month 6	0.591±0.037	0.449±0.034
Wynn et al., 2009		
S. CTX (g/L)	Control group (n=15)	Intervention group (n=15)
baseline	0.380±0.115	0.479±0.261
weeks 4	0.389±0.110	0.401±0.234

Predictive statistical analysis was determined using Statgraphics software (Statgraphics Technologies, Inc., The Plains, Virginia).

Below are the results.

The power of the test (1-ÿ) to be obtained (95%) was considered as factors for the calculation of the sample size, i.e. the probability of rejecting the null hypothesis when there is a real difference, the minimum difference between the treatments (control and intervention group), the variability of the measure and finally the level of significance ÿ (5%), i.e. the probability of obtaining a statistically significant difference when in fact there is no difference.

First analysis (Meunier et al., 2005)

Sample-Size Determination

Parameter to be estimated: difference between two normal means

Desired power: 95,0% for difference = 0,142 versus difference = 0,176

Type of alternative: not equal

Alpha risk: 5,0%

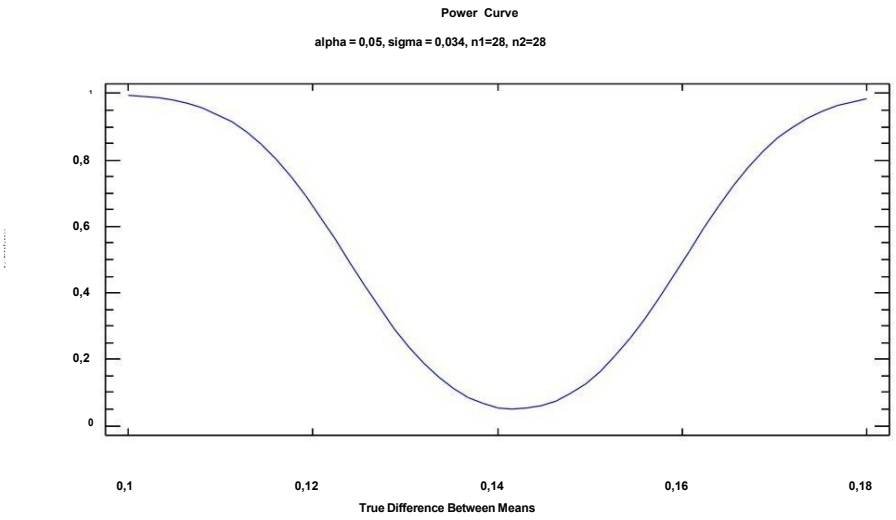
Sigma: 0,034 (to be estimated)

The required sample size is 28 observations from sample 1 and 28 observations from sample 2.

The StatAdvisor

This procedure determines the sample size required when comparing the means of two normal distributions. Assuming that the standard deviations of the normal distributions equal 0,034, 28 observations are required in sample 1 and 28 in sample 2 to have a 95,0% chance of rejecting the hypothesis that mu1-mu2=0,142 when the true mu1-mu2=0,176 (using a two-sided test).

The result estimates that 28 subjects are needed for each group to be enrolled (control and intervention group), in order to ensure a statistical power of 0.95 with an alpha=0.05, using a two-tailed t-test.



For our study we assumed to recruit 30 subjects for each group (the results of the statistical analysis suggest 28 subjects for each group). Therefore, always using these available data in terms of mean, standard deviation and difference between treatments, we evaluate the power of the study at 95% confidence.

Parameter to be estimated: difference between two normal means

Sample sizes: 30 and 30

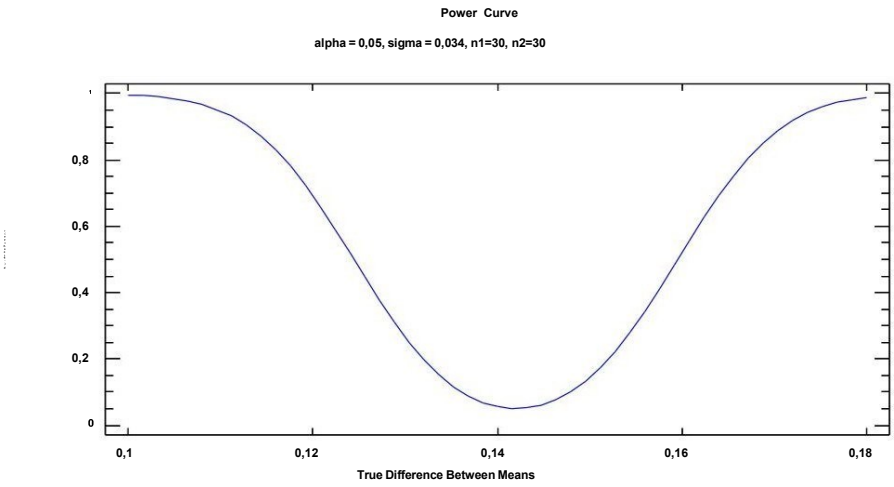
Confidence level: 95,0%

Sigma: 0,034 (to be estimated)

The tolerance for the difference will be +- 0,0175726

The StatAdvisor

This procedure determines the sample size required when comparing the means of two normal distributions. Assuming that the standard deviations of the normal distributions equal 0,034, 30 observations in the first sample and 30 in the second sample will estimate the true mu1-mu2 to within +/-0,0175726 with 95,0% confidence.



Second analysis (Wynn et al., 2009)

The same procedure is repeated using the data of the second scientific article cited.

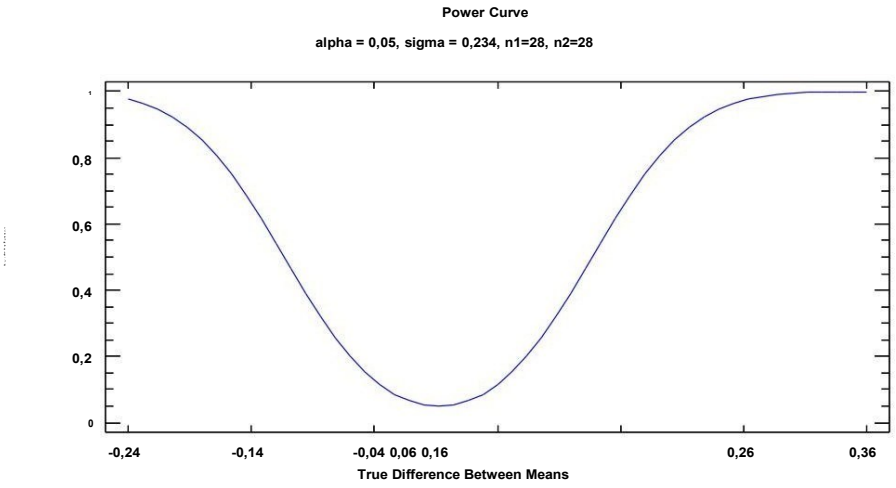
Sample-Size Determination

Parameter to be estimated: difference between two normal means
Desired power: 95,0% for difference = 0,012 versus difference = 0,246
Type of alternative: not equal
Alpha risk: 5,0%
Sigma: 0,234 (to be estimated)

The required sample size is 28 observations from sample 1 and 28 observations from sample 2.

The StatAdvisor

This procedure determines the sample size required when comparing the means of two normal distributions. Assuming that the standard deviations of the normal distributions equal 0,234, 28 observations are required in sample 1 and 28 in sample 2 to have a 95,0% chance of rejecting the hypothesis that mu1-mu2=0,012 when the true mu1-mu2=0,246 (using a two-sided test).



Parameter to be estimated: difference between two normal means

Sample sizes: 30 and 30

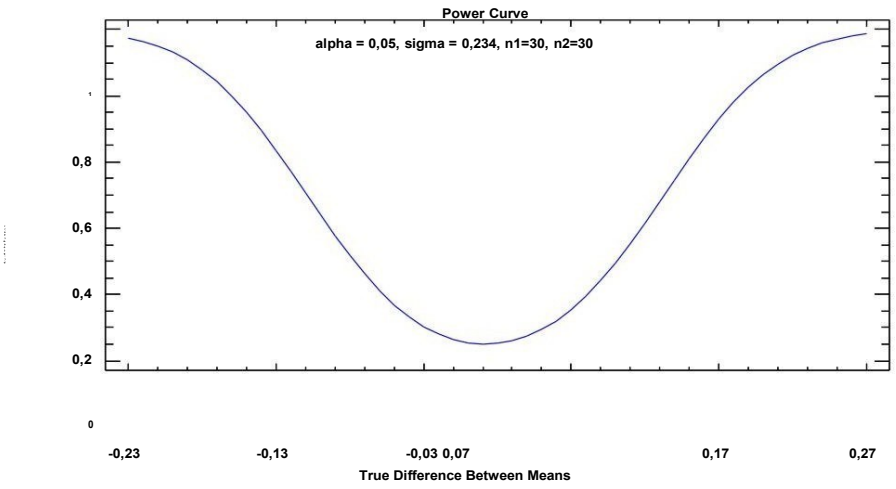
Confidence level: 95,0%

Sigma: 0,234 (to be estimated)

The tolerance for the difference will be +- 0,120941

The StatAdvisor

This procedure determines the sample size required when comparing the means of two normal distributions. Assuming that the standard deviations of the normal distributions equal 0,234, 30 observations in the first sample and 30 in the second sample will estimate the true mu1-mu2 to within +/-0,120941 with 95,0% confidence.



Both statistical analyzes agree with our choice to recruit if possible 30 subjects (instead of 28) for each group (control and intervention group), assuming an extra quota for a probable drop-out. The power of the study considered 95% is high and this increases the necessary number of patients to be enrolled. During the first analysis (data from Meunier et al., 2005), as the power of the study increases, the number of subjects for each group increases, namely:

power 80% 17 subjects;

power 85% 19 subjects;

power 90% 23 subjects;

power 95% 28 subjects.

In the absence of a pilot study, information from the scientific literature had to be used a priori.