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Review Article

A Practical Methodological Primer on Sample Size Determination Using G*Power for Biomedical Research

Vipin Patidar[1], Latika Sinha[2], Suresh Kumar Sharma[3], Rakhi Gaur[1], Saurabh Varshney[4], Shiv Kumar Mudgal[1*]

[1]College of Nursing, All India Institute of Medical Sciences, Deoghar, Jharkhand, India, [2] Department of Community Medicine, Govt. Medical College, Pali, Rajasthan, [3]College of Nursing, All India Institute of Medical Sciences, Jodhpur, Rajasthan, India, [4]Department of ENT, All India Institute of Medical Sciences, Rishikesh, Uttarakhand, India.

Abstract

An appropriate sample size is essential to determine methodological steps that underpin the scientific validity, ethical integrity, and practical feasibility of biomedical and health research. Inadequate estimation may lead to underpowered studies or unnecessary resource use and participant exposure. This article provides a practical overview of statistical power analysis and sample size determination using the G*Power software (version 3.1.9.7), which is a freely available tool for researchers.

The Core statistical concepts, including hypothesis formulation, effect size estimation, significance level (α), statistical power ($1-\beta$), and their dynamic interrelationship, are outlined. The article further describes the G*Power software ecosystem, its graphical user interface, supported statistical test families, and the distinct modes of power analysis. Step-by-step methods with realistic research scenarios demonstrate sample size calculation for commonly used statistical tests, including independent and paired t-tests, z-tests for proportions, one-way ANOVA, correlation analysis, and multiple linear regression. The article also highlights common pitfalls, limitations of G*Power, and advanced considerations in sample size estimation. This article aims to provide researchers with a structured framework for accurate sample size estimation, thereby enhancing study quality, reproducibility, and ethical responsibility in biomedical research.

Keywords: Biomedical research, Effect size, G*Power, Power analysis, Sample size determination.

***Correspondence:** Dr. Shiv Kumar Mudgal, College of Nursing, All India Institute of Medical Sciences, Deoghar, Jharkhand-814152, Mobile no- +919068095488, Email: peehupari05@gmail.com

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Introduction

In an ideal research setting, studying the entire population of interest would yield the most precise and unbiased results. However, in most real-world scenarios, investigating the whole population is neither practical nor feasible due to limitations related to time, cost, logistics, and ethical considerations [1]. Consequently, researchers rely on sampling techniques to select a subset of individuals that adequately represents the target population. Data obtained from these samples are then analyzed to draw valid inferences and estimate population parameters [2]. For such inferences to be meaningful and reliable, careful attention must be paid to selecting an appropriate sample size capable of answering the research question effectively [3].

Sample size refers to the number of subjects, participants, patients, or experimental units included in a study. The systematic process used to decide how many observations should be included is known as sample size determination [4]. This methodological decision is central to the scientific integrity, credibility, and ethical justification of any empirical investigation. In biomedical, psychological, and social science research, determining the correct sample size is not merely a technical requirement but a cornerstone of sound scientific practice that balances statistical rigor with ethical responsibility and practical feasibility [5].

An appropriately calculated sample size ensures that a study has sufficient statistical power to detect clinically or scientifically meaningful effects, while avoiding unnecessary use of resources or exposure of participants to avoidable risk. An "appropriate" sample size is one that is neither too small nor excessively large. Recent methodological guidelines emphasize that high-quality research depends on accurate estimation of effect sizes and adequate statistical power [6].

Scientifically, underpowered studies are prone to Type II errors, resulting in false-negative findings that undermine the validity of conclusions [5-7]. From an ethical perspective, it is unjustifiable to conduct studies involving human or animal subjects if the design lacks the statistical capacity to address the research objectives. Economically, improper sample size estimation can lead to wastage of costly reagents, experimental animals, drug compounds, and valuable laboratory time [8].

In the context of biomedical and pre-clinical research, sample size determination represents a scientific, ethical, and economic imperative in biomedical research. An inadequately powered study exposes subjects to potential harm without generating reliable knowledge, while an excessively powered study can lead to wastage of costly reagents, experimental animals, drug compounds, and valuable laboratory time [9].

Against this background, tools for statistical power analysis and sample size estimation play a critical role in research planning. Today, there are many internet URL links or sample size calculation software available, such as PASS, STATA, R (pwr package), and nQuery, but G*Power provides a free, beginner-friendly interface. This article offers a comprehensive overview of using G*Power (latest ver. 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany; http://www.gpower.hhu.de/), which is freely available software for power and sample size calculation, with a specific focus on applications in biomedical research [9-11]. Unlike existing tutorials, this article not only provides stepwise guidance for commonly used statistical tests but also integrates methodological considerations, practical decision-making frameworks, and common pitfalls encountered during sample size determination. This article is primarily intended for early-career researchers, postgraduate trainees, and clinicians with basic statistical knowledge who require practical guidance in sample size estimation.

Core Statistical Principles for Power Analysis/ sample size estimation

Power analysis summarizes how a research question is converted into a specific sample size needed. Four interconnected statistical parameters are necessary for this conversion.

a. **Hypothesis** The first step in conducting a rigorous a priori power analysis is to translate the research question into explicit statistical hypotheses. This process defines the parameters to be tested and determines the appropriate statistical model within G*Power.

The null hypothesis (H_0) represents the assumption of no effect, no difference, or no association between the study variables. For example, there is no statistically significant difference in mean blood pressure reduction between Drug A and placebo. In hypothesis testing, the analytical objective is to evaluate evidence against H_0 [7].

The alternative hypothesis (H_1) represents the presence of a true effect or difference and forms the basis for power and sample size estimation.[7] In G*Power, H_1 must be specified as either two-tailed (non-directional) or one-tailed (directional):

• Two-tailed (non-directional) hypothesis: This is the default and most commonly selected option in biomedical research. It tests for any difference between groups without specifying the direction of the effect (e.g., the effect of Drug A differs from that of placebo).

• One-tailed (directional) hypothesis: This tests for an effect in a pre-specified direction and requires strong a priori justification based on existing evidence or biological plausibility (e.g., Drug A produces a greater reduction in blood pressure than placebo). One-tailed testing increases statistical power for detecting effects in the specified direction but does not assess effects in the opposite direction.

b. **Effect Size**

Effect size is a standardized measure of the magnitude of a phenomenon. It is perhaps the most important and difficult parameter to define since it connects statistical computation to practical significance.[8] It measures the strength of a relationship or the extent of the difference between groups, regardless of sample size. Because the true effect size is unknown prior to the study, it must be estimated. Thus, the effect size can be estimated using the following recommended methods:

- Empirical Data: Incorporate results from pilot research or a previous study covering the same results in similar populations.
- Clinical/Meaningful Difference: Calculate the smallest change in an outcome that a patient or clinician would consider helpful, known as the Minimal Clinically Important Difference (MCID), and convert it into a statistical effect size.
- Conventional Values: Use conventions particular to the field of study, such as Cohen's benchmarks for different statistical tests given in Table 1 [12].

Table 1: Cohen's d value for different statistical tests

Statistical test	**Effect size measure**	**Small (S)**	**Medium (M)**	**Large (L)**
Independent t-test	Cohen's *d*	0.20	0.50	0.80
Dependent (paired) t-test	Cohen's *dz*	0.20	0.50	0.80
One-way/factorial ANOVA	Cohen's *f*	0.10	0.25	0.40
Correlation (Pearson/Spearman)	Correlation coefficient *r*	0.10	0.30	0.50
Linear regression (overall model)	Cohen's f^2	0.02	0.15	0.35
Chi-square test	Cohen's *w*	0.10	0.30	0.50

c. Error Management: Balancing Type I (α) and Type II (β) Errors

When population-level inferences are made based on sample data, two types of statistical decision errors may occur. A priori power analysis, as implemented in G*Power software, is used to control the probabilities of these errors during study planning.

A Type I error (α) is defined as the probability of rejecting the null hypothesis (H_0) when it is true, resulting in a false-positive finding. The significance level (α) is specified by the investigator and represents the acceptable risk of this error. The confidence level ($1 - \alpha$) reflects the probability that the confidence interval contains the true population parameter.

A Type II error (β) is the probability of failing to reject the null hypothesis when it is false, leading to a false-negative result. Statistical power, defined as $(1-\beta)$, represents the probability of correctly detecting a true effect of a specified effect size at the chosen α level. Conventionally, studies are designed with 80% power (β = 0.20), implying a 20% probability of Type II error. 90% or 95% power is sometimes needed for high-risk confirmatory research such as pivotal drug studies. In G*Power, power is set a priori (commonly 0.80 or 0.90) to determine the required sample size.

d. The Dynamic Relationship: There is a dynamic balance between the four parameters that is effect size, power (1-β), significance level (α), and sample size (N). In power analysis, any one parameter can be estimated when the remaining three are specified. For example, you need to increase α, decrease power, or increase sample size in order to detect a smaller effect size. Power analysis software such as G*Power utilizes this relationship to estimate sample size or effect size.

The G*Power Software Ecosystem

G*Power is a widely used free software tool developed at Heinrich Heine University for conducting statistical power analysis. It has become a vital tool in the fields of biomedical, social, and behavioral sciences, providing an easy-to-use interface for carrying out statistically demanding power analyses that successfully bridge the gap between complex statistical theory and real-world research needs by providing an extensive feature set contained in an intuitive graphical user interface (GUI) [10].

a. Overview of Statistical Tests Supported

One notable feature of G*Power is the broad range of widely used statistical techniques. The researchers can choose the best analytical approach for almost any study design, which are methodically grouped into logical "test families" (Figure 1A). The t-test family includes independent, paired (dependent), and one-sample designs to effectively perform mean comparisons. The F-test family includes many regression models and variance analyses (ANOVA), including one-way, factorial, and repeated measurements. The χ^2 (Chi-square) test family includes independence tests in contingency tables and goodness-of-fit evaluations for categorical datasets. The z-test family provides tools for proportion analysis, making it easier to compare one or two independent proportions. Additionally, G*Power incorporates exact tests designed for non-parametric scenarios based on Poisson, binomial, and other discrete distributions.

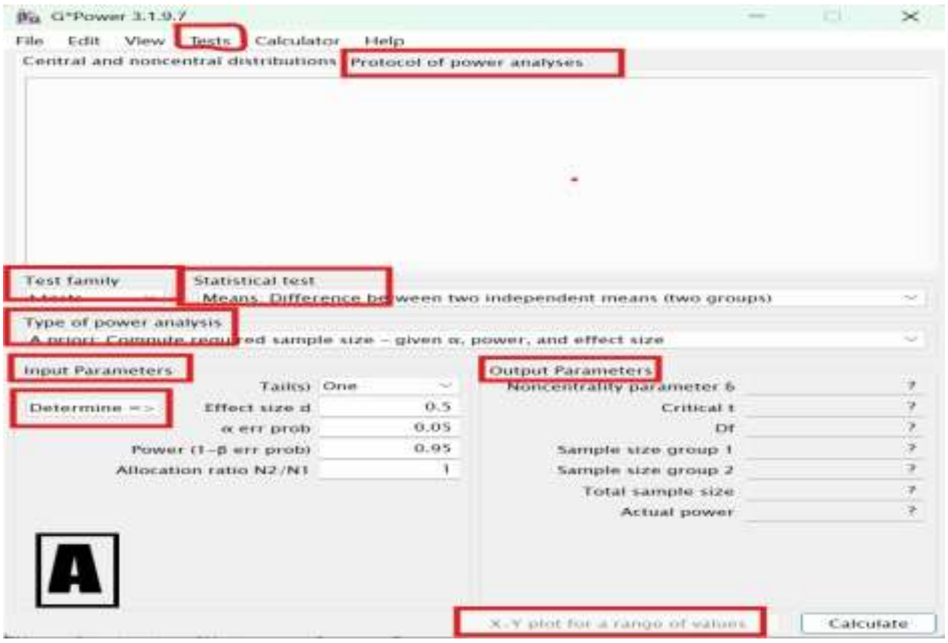

Figure 1(A). Graphical user interface (GUI) of G*Power software showing key options at the main window.

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b. Navigating the Graphical User Interface

The G*Power interface is carefully designed to promote an orderly workflow. The primary window reveals a number of important sections when it first opens. The drop-down menus, like Statistical test" and "Test family," allow the user to select the appropriate test and analysis type. The "Type of power analysis" menu, which appears immediately below, specifies the researcher's purpose for the computation. For example, selecting a sample size. The "Input Parameters" section, which is located in the central portion of the screen, allows users to enter values for effect size, alpha, power, and sample size. The key option in this scenario is "Determine =>," which triggers a specialized calculator to transform raw data, such as means and standard deviations from a preliminary study, into the necessary effect size help to reduce human error. The computation results are then shown in the "Output Parameters" box. Additionally, tabs like "Protocol of power analyses" at the top of the screen maintain a record of every calculation that is performed, which is essential for reporting and documentation. The option "X-Y plot for a range of values" button makes it easier to create graphs that show how parameters relate to one another, as well as how power and sample size are correlated. Figure 1(A) shows a graphic representation of this logical interface and its main functional areas.

c. The Five Analysis Modes

There are five discrete analytic modes (A Priori, Post-Hoc, Sensitivity, Criterion, and Compromise) that G*Power provides (select type of power analysis dropdown menu), and each of which addresses a different research question. The standard for study planning is the "A priori" analysis that is used before data is collected, which determines the required sample size (N) based on the desired power, alpha level, and expected effect size. The second mode, a "post hoc" analysis, is carried out after a study is finished, which uses the collected sample size, the observed effect size, and alpha to determine the obtained power that could help to understand non-significant results. The question, "What effect could I realistically find?" is addressed with another mode, a "Sensitivity" analysis, which is a potent planning tool that establishes the minimum detectable effect size at given fixed alpha, power, and feasible sample size. The "Criterion" analysis, which is helpful in certain methodological contexts, determines the necessary alpha level given at fixed effect size, power, and sample size. Lastly, the "Compromise" analysis offers an appropriate answer for situations with limited resources by enabling the simultaneous modification of both alpha and beta error probability while the sample size is fixed. A comparative summary of these five distinct analysis modes is shown in Figure 1(B).

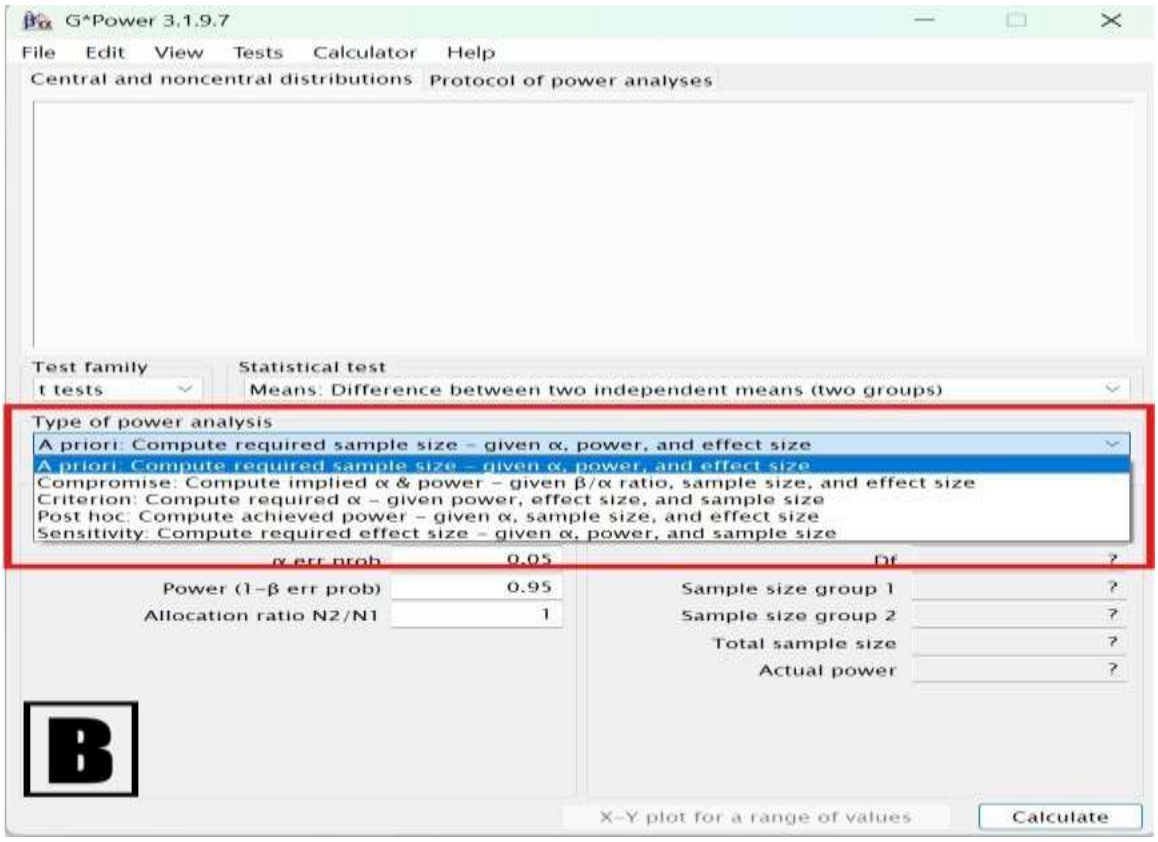

Figure 1(B). Graphical user interface (GUI) of G*Power software showing the five analysis modes.

Strategic Framework for Sample Size Determination
We can consider the process of calculating sample size as equal to building a house, which requires a thorough plan (study goals or objectives), suitable instruments (statistical method), accurate measurements (parameters), and an efficient arrangement of space (allocation).

a. Primary outcome and hypothesis: It is essential to have a clear understanding of the phenomenon before using the software, such as defining the primary outcome and formulating the hypotheses being studied.

b. Identify the Appropriate Test: G*Power helps us to choose a statistical test using two different approaches.

- Choose according to the research design: Use the main "Tests" menu at the top of the window and explore its alternative options in the same way that would explain the study. For example, in order to compare the mean outcomes of two distinct groups of individuals, you would select: Tests > Means > Two independent groups. The same is shown in Figure 2(A).
- Choosing according to the mathematical distribution: Use the "Test family" dropdown option in the main window to choose a test according to the underlying statistical method. For example, in the "Test family" menu are t-test, F-test, etc.

c. Parameter Specification: Next step is the sources of numerical data where values for Effect Size, Power (1-β), and Significance (α) are determined as seen in Figure 2(B).

- Effect: Avoid making speculative estimates about effect size. The most trustworthy sources are: 1) information from your own pilot experiments; 2) published results from related studies; or 3) a clinically significant change (the minimal improvement that holds relevance for a patient).
- Power and Alpha: These parameters can be determined by conventional standards, which aim for 80% Power with Alpha α = 0.05.

d. Allocation Ratios: Allocation ratio refers to how participants are distributed between study groups. The most effective and efficient design is usually a 1:1 ratio (equivalent group sizes). Choosing a different ratio (like 2:1) will require more participants overall to get the same level of reliability. When you choose a statistical test involving two independent groups in G*Power, like the t-test for two independent means or the z-test for two proportions, you may find the Allocation Ratio in the "Input Parameters" area of the main window, as seen in Figure 2(B).

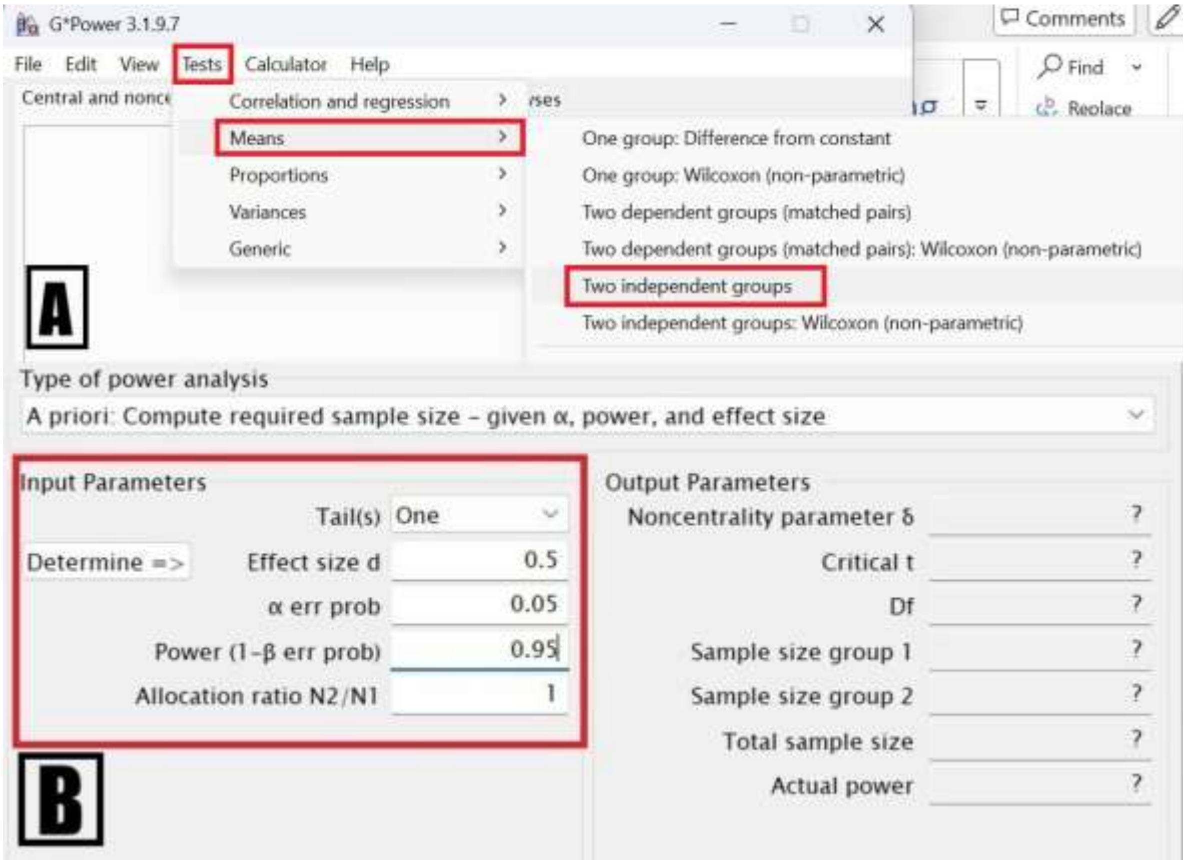

Figure 2. G*Power software showing (A) the selection of statistical test, (B) Details of input parameters, including allocation ratio.

Step-by-Step Tutorials for different tests with scenarios

The first example offers a thorough, step-by-step explanation of each step. The following examples are given briefly, highlighting only important parameters and outcomes to prevent repetition. Effect size estimation follows the principles described earlier (see Table 1).

1. Comparing Independent Groups: The Two-Sample t-Test (Figure 3)

Scenario: We are comparing a new exercise program (Group B) to a standard one (Group A). The primary outcome is the average improvement in a fitness score after 8 weeks, and the goal is to calculate how many total participants we need to recruit (A Priori Power Analysis) [9-10].

Step 1 is to select the Test: In the top menu, click: Tests > Means > Two independent groups, as seen in Figure 3(A).

Step 2 is to set our analysis goal: In the "Type of power analysis" dropdown, select: "A priori: Compute required sample size..." option.

Step 3 is entering the parameters: We can fill in the "Input Parameters" fields in the following steps-

- Tail(s): Two (as we are checking if the programs are different, not specifically if one is better).
- Effect size d: 0.5

  How to get effect size: The standard effect size values of 0.2, 0.5, and 0.8 are provided by G*Power software for small, medium, and large effect sizes, respectively (Table 1). Instead of depending on general conventions, we can use G*Power to get an exact, study-specific effect size when we have published results or preliminary data from a pilot study. The steps are as follows (Figure 3B)-
  - In the main input window, click the "Determine =>" option next to the "Effect size" field.
  - Enter the known means and standard deviations for Groups 1 and 2 after choosing the option for equal or unequal group sizes for a two-group comparison.
  - Then select the "Calculate and transfer to main window" option, and the program automatically fills in the main effect size field with the corresponding effect size.
- α err prob: 0.05 (Standard 5% risk of a false positive).
- Power (1-β err prob): 0.80 (Standard 80% chance to detect the effect).
- Allocation ratio N2/N1: 1 (we plan to have equal group sizes).

Step 4: Calculate the sample size as shown in Figure 3(C)

- Click the "Calculate button" down.
- Look at the "Output Parameters":
  - Total sample size: 128.
  - It means we need 64 participants in Group A and 64 in Group B.

How to report: In order to achieve a medium effect size (*d*=0.5), α=0.05, and Power=0.80, G*Power suggests recruiting 128 participants in total (64 in each group).

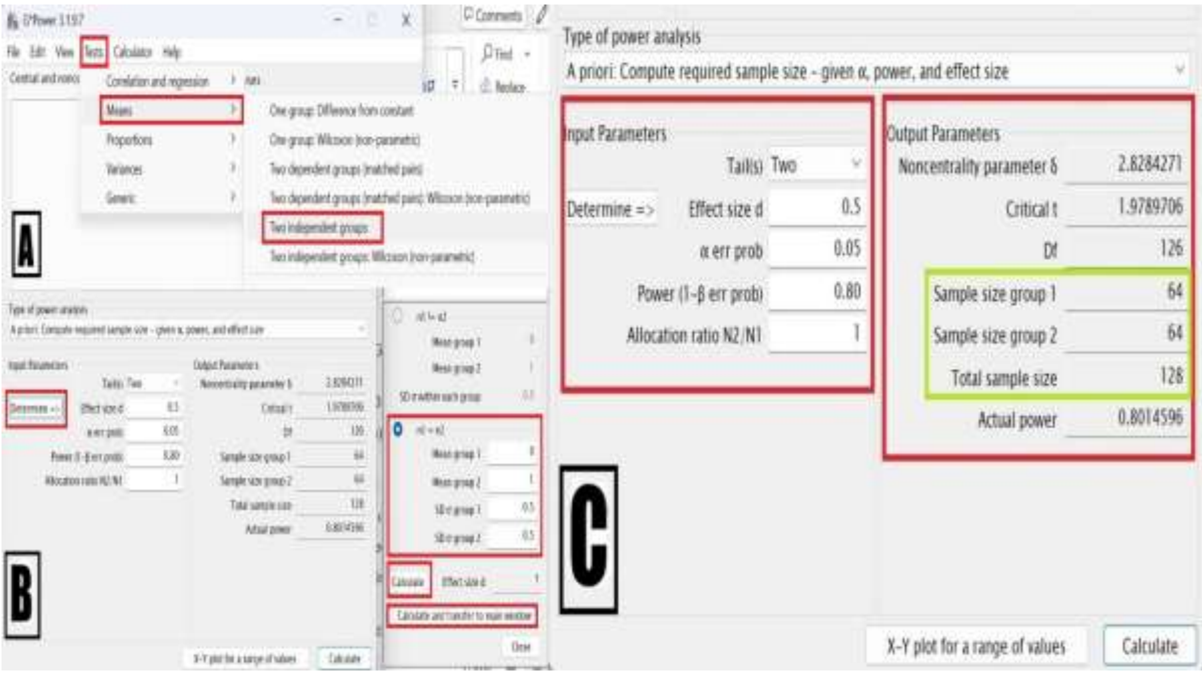

Figure 3. Illustrating a sample size calculation for two independent sample t test. (A) selection of statistical test, (B) effect size estimation using the determine option, and (C) Input and output parameters.

2. Paired Measurements: The Dependent t-Tests (Figure 4)

Scenario: One researcher is testing a new runner feedback device that will be used to measure the performance of a single group of runners both before and after a six-week training program. The primary outcome is the average improvement in each runner's score from the first test to the second.

- Test: Two dependent groups (matched pairs) as seen in Figure 4.
- Analysis: A priori: Compute required sample size
- Tail(s): Two
- Effect size (dz): 0.5
- α err prob: 0.05
- Power (1-β err prob): 0.80
- Result: Total sample size: 34.

How to report: In order to get a medium within-person effect size (dz=0.5), α=0.05, and Power=0.80, G*Power recommends including 34 participants in total. Every subject is measured twice, once before and once after, providing 34 matched pairs for the study.

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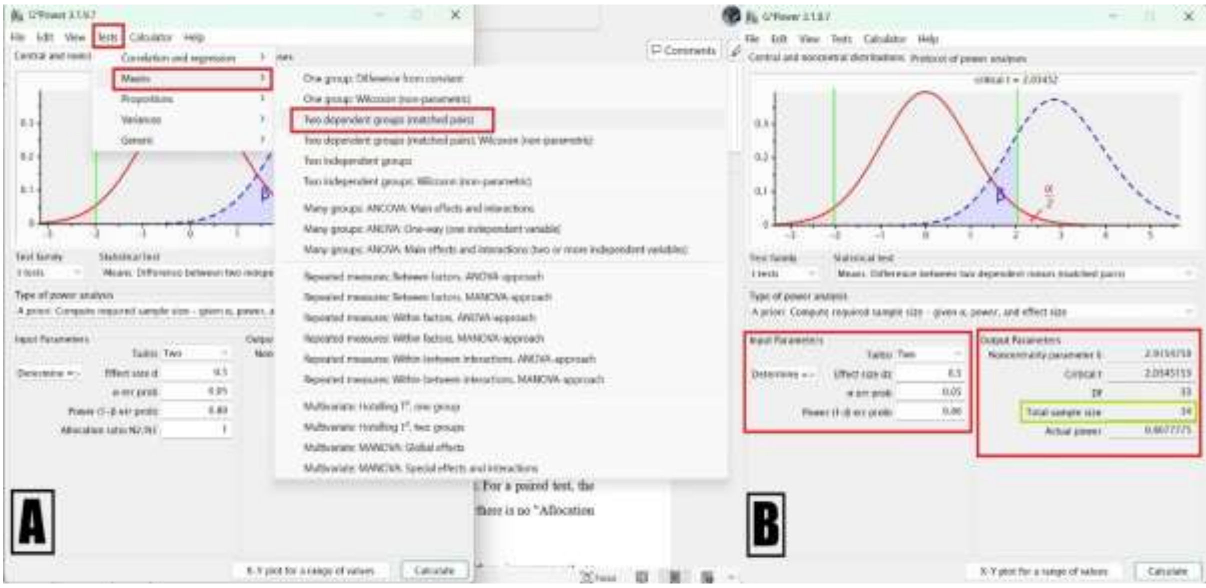

Figure 4. Illustrating a sample size calculation for a dependent t-test. (A) selection of statistical test, (B) Input and output parameters.

3. Comparing Proportions: Z test for Two Independent Groups

Scenario: A common allergy drug's adverse effect rates are being compared by a researcher between two alternative formulations. The primary outcome is the proportion of patients who report feeling drowsiness 24 hours after taking the drug.

- Test: Two independent groups: Inequality, z-test as shown in 5.
- Analysis: A priori: Compute required sample size
- Tail(s): Two (we want to detect if the drowsiness rate is different, not just lower).
- Proportion (p1): 0.15
- Proportion (p2): 0.25
- α err prob: 0.05
- Power (1-β err prob): 0.80
- Allocation ratio N2/N1: 1
- Result: Total sample size: 500 (250 in each group)

How to report: In order to get a significant difference between a 15% and a 25% drowsiness rate at α=0.05, and Power=0.80, G*Power recommends including 500 participants in total (250 per group).

4. Multiple Group Comparisons: One-Way ANOVA

Scenario: A dietitian is evaluating how well three distinct diet plans, Plan A: Low-Carb, Plan B: Mediterranean, and Plan C: Calorie-Restricted, affect weight loss during a 12-week period. One of the three plans will be chosen at random for each participant. The average weight loss (in kg) for each participant at the end of the research is the primary outcome.

- Test: Many groups: ANOVA: One-way (one independent variable)
- Analysis: A priori: Compute required sample size
- Effect size f: 0.25 (If we have pilot data with mean values and standard deviations for each group, we may determine the precise effect size rather than relying on a general convention).
- α err prob: 0.05
- Power (1-β err prob): 0.80
- Number of groups: 3
- Result: Total sample size: 159 (53 participants in each group).

How to report: In order to get a medium effect size (*f*=0.25), α=0.05, and Power=0.80 across 3 groups, G*Power recommends a total of 159 participants. This would mean aiming to enrol approximately 53 participants in each diet group.

5. Pearson Correlation Analysis

Scenario: A public health researcher is examining the effect of moderate exercise hours per week on HDL cholesterol levels in individuals, with the primary outcome to see if there is a significant linear correlation between these two variables.

- Test: Correlation: Bivariate normal model
- Analysis: A priori: Compute required sample size
- Tail(s): Two
- Correlation ρ H1: 0.3 (If we have pilot data or published results with the correlation coefficient (r) or the coefficient of determination (r^2), we may determine the precise effect size.
- α err prob: 0.05
- Power (1-β err prob): 0.80
- Correlation ρ H0: 0 (The null hypothesis is always that there is no correlation).
- Result: Total sample size: 84

How to report: In order to get a medium correlation (ρ=0.3), α=0.05, and Power=0.80, G*Power recommends a total of 84 participants.

6. Regression analysis

Scenario: A researcher wants to develop a model to predict an individual's systolic blood pressure using three important predictors: body mass index (BMI), average daily sodium intake (mg), and age (years) with the primary outcome to determine whether the combined predictors account for a significant amount of the variance in blood pressure (R2 > 0) and whether the total regression model is statistically significant.

- Test: Linear multiple regression: Fixed model, R^2 deviation from zero
- Analysis: A priori: Compute required sample size
- Effect size (f^2): 0.10 (The "Determine =>" option is also utilized for regression analysis, as we need to enter the known R^2 or the detailed variance structure of the predictors from previous literature.
- α err prob: 0.05
- Power (1-β err prob): 0.80
- Number of predictors: 3 (For BMI, sodium intake, and age).
- Result: Total sample size: 114

How to report: In order to get a small-to-moderate effect size (f2=0.10) with α=0.05, and Power=0.80, G*Power recommends a total of 114 participants.

Advanced Considerations in Sample Size Calculation

Many of the advanced factors must be considered to ensure precise and realistic sample size estimation in biomedical research beyond the basic power analysis. Researchers should attribute for anticipated loss to follow-up by estimating the calculated sample size accordingly. In various studies that used cluster sampling or multicenter designs, adjustment for the design effect is essential to address intra-cluster correlation. Additionally, when multiple outcomes or repeated statistical testing are planned for any study, appropriate corrections like Bonferroni adjustment must be considered to control Type I error inflation. Other factors, including handling of missing data, non-normal distributions, and potential confounders, should also be taken into consideration during the planning phase.

Common Pitfalls and Misinterpretations

The common issue is the inappropriate selection of effect size without adequate justification, which can lead to underpowered or overpowered studies. Additionally, misuse of post-hoc power analysis is another concern as it provides limited interpretive value, which may mislead. Many times, researchers may also overlook key assumptions underlying statistical tests, like normality or independence of observations, that result in inaccurate estimates. Failure to adjust for realistic factors, including attrition rate or unequal group allocation, further limits the applicability of the G*Power software.

Conclusion

Sample size determination is a fundamental element of sound and ethical research design, directly influencing the validity, reliability, and interpretability of study findings. Proper statistical power analysis helps balance the risks of Type I and Type II errors while ensuring efficient use of resources and responsible involvement of research participants. This article provides a structured approach to sample size determination using G*Power. By emphasizing clear hypothesis formulation, realistic effect size estimation, and appropriate selection of statistical tests, researchers can strengthen study planning and reproducibility. Integrating systematic power analysis into research protocols ultimately enhances scientific rigor and supports the generation of credible and meaningful evidence in biomedical research.

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