John Graunt
(24 April 1620 – 18 April 1674)

His book *Natural and Political Observations Made upon the Bills of Mortality* (1662) used analysis of the mortality lists of London in the attempt to create a system for warning on the onset and spread of bubonic plague in the city. The system did not properly work, but Graunt's work in studying the lists resulted in the first statistically based estimation of the population of London.

He is credited with producing the first life table, giving probabilities of survival to each age. Graunt is also considered as one of the first experts in epidemiology since his famous book was concerned mostly with public health statistics.

He developed early human statistical methods that later provided a framework for modern demography.

Among several other mathematicians, Blaise Pascal, Pierre de Fermat (and Carl Friedrich Gauss one century later) laid down the foundations of the probability theory mostly studying the games of chance.

Today, statistics is widely employed in government, business, natural and social sciences and BIOMEDICINE.

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**SPESE PER LA «SALUTE»**
*(dati 2008)*

Europa spende in media 8.8% del GDP (gross domestic product)

Italia 8.9%

Spese per la salute **1,100 Miliardi €** per una popolazione al Gennaio 2009 di circa 500 ML di unità

**Fonti:**
OECD – Organization for Economic Co-operation and Development
EFPIA – European Federation of Pharma Industries and Associates
What are Clinical Trials? (I)

- Clinical trials are medical research studies involving people. They aim to test whether different treatments are safe and how well they work. Some trials involve healthy volunteers, others involve patients who may be offered to participate in a trial during their care and treatment. Clinical trials are carried out to answer specific questions about health and disease.

- Well-designed clinical trials are a vital contribution to advancing medical knowledge, in order to improve treatment, care and quality of life for patients.

- Clinical trials aim to find the best ways to:
  - Prevent disease and reduce the number of people who become ill
  - Treat illness to improve survival or increase the number of people cured
  - Improve the quality of life for people living with illness, including reducing symptoms of disease or side effects of therapy
  - Diagnose disease ad health problems

- Interventions may consists of drugs, devices, behavioral intervention etc.

Clinical trials are carried out in stages:

Phase I:
- Small number of healthy people; Aim: safety

Phase II:
- Larger number of healthy people AND patients
  - Aim: safety/side-effects and efficacy (dose-finding)

Phase III:
- Large numbers (100s-1000s) of patients in 1 or several countries
  - Aim: compare the effects of the new drug with placebo or standard* treatment
    - find out how well the drug works and how long the effects last
    - find out about how common and serious any side effects are / longer term risks

Phase IV (after licensing):
- Aim: find out how well the drug works when used more widely
  - long-term risks and benefits
  - find out about possible rare side effects

- MATHEMATICS: quantification
- STATISTICS: comparison

*Pragmatic Trials

Adapted from: Understanding Clinical Trials UKCRC 2006 – www.ukcrc.org
Esempi nel settore del Diabete

Normal glucose tolerance depends upon an adequate control of Islet Hormones

Reduced insulin action leads to Hyperglycemia

Insulin Resistance

“a state (of a cell, tissue, system or body) in which greater-than-normal amounts of insulin are required to elicit a quantitatively normal response”

Berson & Yalow, 1970.
Islet Dysfunction leads to Hyperglycemia

- Fewer β-Cells
- Insufficient Insulin
- α-Cells Hypertrophy
- Excessive Glucagon

↑ Glucose

↓ Glucose uptake

↑ HGO (Hepatic Glucose Output)

β cells are unable to produce enough insulin to lower blood glucose levels

CONSTANTLY HIGH GLUCOSE

Elevated Glucose Leads to Tissue Damage in Many Organ Systems with Serious Long-Term Complications

- Eyes (retinopathy, glaucoma, cataracts)
- Brain and Cerebral Circulation (stroke, TIA)
- Kidneys (nephropathy, ESRD)
- Heart and Coronary Circulation (angina, MI, CHF)
- Peripheral Nervous System (peripheral neuropathy)
- Peripheral Vascular Tree (peripheral vascular disease, gangrene, amputation)

CHF = Congestive Heart Failure; ESRD = End-Stage Renal Disease; MI = Myocardial Infarction; TIA = Transient Ischemic Attack

Insulin Resistance

Quantification of insulin resistance is attained by measuring insulin sensitivity.

An index of Insulin Sensitivity quantifies the insulin action of inhibiting endogenous glucose production from the liver and promoting peripheral glucose utilization by muscle and fat.

Insulin Sensitivity

Change of glucose disappearance (following a glucose load) for an unit change of systemic insulin concentration.

\[
S_I = \left( \Delta \text{Gluc Disapp} / \Delta \text{Ins} \right)_{\text{iss}}
\]

NOTE

for calculating \( S_I \) it is necessary to introduce a mathematical relationship (or a model) between glucose disappearance and insulin concentration
Results are statistically evaluated with ANOVA; comparisons were performed both within the respective groups before versus after 52 weeks of treatment and between the two groups.

Probability level of random difference between groups: * p<0.05

*Insulin sensitivity in young (type 2, obese and control subjects)
## Insulin Sensitivity in NASH

**NASH (N=19)**
- BMI = 26 ± 1 kg/m²
- Age = 38 ± 2 years

**Control (N=19)**
- BMI = 25 ± 1 kg/m²
- Age = 37 ± 2 years

![Graph showing insulin sensitivity index comparison between NASH and Control](image)

*Pagano et al: Hepatology, 2002*

Patients with end-stage renal disease exhibit elevated insulin resistance.

“Normal” glucose disappearance is attained because of an elevated insulin secretion.

### Insulin Sensitivity Index

![Graph showing insulin sensitivity index in HD patients before and after vitamin D treatment](image)

Calcitriol normalizes insulin sensitivity in non-diabetic patients with end-stage renal disease.

Insulin secretion (still more elevated than in control subjects) is slightly reduced with active vitamin D.

### IVGTT in HD patients vs. Control subjects

- Creatinine (mg/dl) 11.5±1.1 in HD; 0.9±0.01 in CNT (p<0.0001)
- Insulin Sensitivity Index
  - p<0.002

-Creatinine (mg/dl) 11.5±1.1 in HD; 0.9±0.01 in CNT (p<0.0001)

- Insulin Sensitivity Index
  - p<0.002

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**VINCOLI**

Dati normalmente distribuiti; se no → log transformation

Opportuno fare quindi a priori un test di normalità

Il numero di soggetti – se possibile – andrebbe deciso a priori con la power-analysis:

\[
N = \left[ \frac{Z_\alpha \sigma_0 + Z_\beta \sigma_1}{\mu_1 - \mu_0} \right]^2
\]
Glucose is injected (0.3 g/kg BW) and glucose and insulin concentrations are measured for 3 h.

**IVGTT and the Minimal Model**

Glucose production (liver)

Glucose utilization (muscle, fat)

**Derivation of the Insulin Sensitivity Index ($S_i$)**

\[
\frac{dG}{dt} = (p_1 - X_p)G + p_4 \\
\frac{dX_t}{dt} = 0 = p_2 X_t + p_3 I_t,
\]

Substituting for $X_t$, we obtain

\[
\frac{dG}{dt} = [p_1 + (p_3/p_2)M]G + p_4.
\]

Glucose effectiveness is given as $-\frac{(dG/dt)}{G}$, that is

\[
E_t = \frac{p_3}{p_2} I_t - p_t.
\]

Insulin sensitivity ($S_i$) is then the dependence of $E$ (evaluated at a specific insulin concentration) upon insulin, that is $\frac{dE_t}{dI_t}/E_t$, or

\[
S_i = -\frac{p_3}{p_2}.
\]
**VALIDATION STRATEGY**

- **Direct Measurement** → **Gold Standard** → **Calculated Results** → **Mathematical Model** → **Experiment (Test)**

- **MINIMAL MODEL**
  - Validation
  - **GOLD STANDARD**
    - glucose clamp

- **Calculated Results**
  - **OK**?

- **MINIMAL MODEL**
  - MM output
  - **GOLD STANDARD**
    - glucose clamp

- **INSULIN SENSITIVITY FROM IVGTT**
  - **simplified formula**
    \[
    CS_t = \frac{1}{t_4 - t_3} \int_{t_3}^{t_4} (I(t) - I_0) \, dt
    \]
  - **Units:** min\(^{-1}\)/(µU/ml)

  - **A. Tura, S. Sbrignadello, G. Pacini. Diabetologia 2009**
Simplified formula for insulin sensitivity from (short) IVGTT

\[ CS_I = 0.3 \times S_I \]

N=144, \( r=0.934, p<0.0001 \)

\[ CS_I = 0.3 \times S_{IMM} \]

Relationship between computed (\(CS_I\)) and minimal model estimated (\(S_{IMM}\)) insulin sensitivity in control subjects of various age and weight

Slope = 0.97, \( p > 0.2 \) vs. slope=1; \( r = 0.907, p < 0.0001 \)
**Relationship between** \( CS_I \) [normalized with the factor 0.3 \( \text{of CNT} \)] and \( S_I \text{MM} \) in 55 subjects with renal disease

![Graph showing the relationship between \( CS_I \) and \( S_I \text{MM} \).](image1)

Slope = 0.88, \( p > 0.1 \) vs. slope=1; \( r = 0.893, p < 0.0001 \)

**Comparison between** \( CS_I \) and Clamp M in normo glucose tolerant (circles), impaired tolerant (squares) and diabetic (triangles) subjects

![Graph comparing \( CS_I \) and Clamp M.](image2)

regression lines are virtually equivalent to the identity line

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**CONCLUSIONI**

- Individuazione del problema
- Scelta del numero corretto di soggetti/esperimenti
- Corretta osservazione dei dati (normalità, skewness, statistica descrittiva, outliers, ...)
- Scelta del test più opportuno (possibilmente semplice e di facile comprensione)
- Uso del software appropriato
- Interpretazione dei risultati sulla base della specifica situazione/condizione del «sistema» in studio

"There is no statistical test, however intuitive and simple, which will not be abused by medical researchers . . . just to get \( p < 0.05 \) !!!!!"

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**Bland–Altman plot**

![Bland–Altman plot](image3)

only a few samples were outside the limits for equivalence

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A. Tura, S. Sbrignadello, G. Pacini. Diabetologia 2009
This approach is used to analyze the performance of a screening tests considering a reference standard.

True-positive rate (sensitivity) vs. false positive rate (100 – specificity) is plotted for each measurement.

The estimated area under the fitted smooth curve ranges from 0.5 (no apparent accuracy) to 1.0 (perfect accuracy) as the ROC curve moves toward the left and top boundaries of the ROC graph.

The point nearest to the intersection of the curve with the 100%-to-100% diagonal represents the best equilibrium between sensitivity and specificity (point of equilibrium).

Evaluation of Fasting Capillary Blood Glucose (FCG) as Screening Test for Diabetes

The **sensitivity** is how good FCG is at picking out patients with diabetes and gives us the proportion of cases picked out by the test, relative to all cases who actually have diabetes.

The **specificity** is the ability of the test to pick out patients who do NOT have the disease.

<table>
<thead>
<tr>
<th>FCG (mmol/l)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetes</td>
<td>IGT</td>
</tr>
<tr>
<td>3.9</td>
<td>97.1</td>
<td>94.4</td>
</tr>
<tr>
<td>4.4</td>
<td>95.4</td>
<td>83.9</td>
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<td>92.0</td>
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<td>43.0</td>
<td>2.6</td>
</tr>
<tr>
<td>8.9</td>
<td>37.9</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Data are %.

Overall sensitivity of 87.2% using 5.6 mmol/l FCG as cutoff for the diagnosis of diabetes is observed.

The cutoff point 5.6 mmol/l has a sensitivity for diabetes of 87.2% and a specificity of 72.4%.
Detecting GDM

Normal FBG vs. FBG pre-test + risk score