

Effects of the ingestion of human, cow and cow-modified milk, on glucose, amino acid and hormone responses in humans
Acronym: PROLAT
(extract)

Objects of the study:

To evaluate the effects of the ingestion of human and cow milk, as well as of protein-modified cow milk, on glucose, amino acid and hormone responses (insulin, glucagon, IGF-1, incretins) in humans.

Background

Milk is the first food for the development of the neonate [...] and a key nutritional substrate also over the entire human life [...]. The nutritional importance of milk (1-5) is due to its balanced content in both essential and non essential nutrients, such as:

- High biological value proteins, rich in essential amino acids;
- Lipids
- Lactose
- Minerals
- Vitamins
- Energy

Furthermore, milk provides probiotics and other functional substances [...] and is a key stimulator of hormone secretion favoring nutrients' utilization.

[...], Among the stimulated hormones, the most important ones are insulin, glucagon, IGF-1, and the incretins GLP-1 and GIP (1). Milk lactose and proteins have a key role in the stimulation of their secretion [...] (2, 3). Human milk, despite its low protein content, exhibits a powerful effect on insulin secretion (4) [...].

Species-specific differences in milk composition.

Milk from various species besides the human one, can be used in human nutrition, cow, sheep, goat and donkey milk [...].

There are important species-specific differences in milk [...]. For instance human milk, as compared to cow milk, is moderately hypercaloric, hyperglucidic, markedly ipoproteic, isolipidic, richer in iron and relatively poorer in salts and oligoelements (4).

The qualitative composition of the protein fraction is also different between human and cow milk [...]. The effects of such a different protein composition on hormone secretion is not known.

[...]

Human milk apparently is endowed with specific effects on hormone secretion [...]. The factors underlying such effect are not yet known (5).

The aims of the study are therefore the following:

1. To determine glucose, amino acid, and hormonal responses to natural human and cow milk at strictly-controlled, iso-lactose and iso-volume conditions.
2. To determine the same responses to either human or cow milk at modified protein concentrations. [...].

Experimental design:

- Recruitment of healthy volunteers of both sexes (age between 20 and 60 yrs, BMI between 18.5 e 25 Kg/m²),
- A written consent form will be signed.
- The milk load(s) will be administered after the overnight fast.
- At least one week will be allowed between each test in the same volunteer.

The following milk type will be randomly administered to the volunteers:

1. Human natural milk
2. Cow natural milk
3. A low-protein, cow-derived milk, similar to the human one for total protein content, but with a casein-to-whey protein ratio of casein typical of human milk (20:80);
4. A high-protein, cow-derived milk, similar to natural cow milk for total protein content, but with a casein-to-whey protein ratio of casein typical of human milk (20:80);

All milk loads will contain the same lactose amount (0.36 g/kg BW)

The human milk, whenever available, will be furnished by the Milk bank of the Dept. of Pediatrics of Padova University Hospital or by other available official Milk banks [...].

All modified milk types will be handled and prepared under proper aseptic conditions.

Study protocol (see also the attached scheme)

The volunteers were admitted to the clinical study unit at 08:00 after the overnight fast. A 20-g cannula was inserted in an antecubital vein for blood withdrawal. After two baseline samples spaced by $\approx 10'$, the milk load was administered over 2'-5' to the volunteers. Starting from the end of milk ingestion ($t = 0'$), blood samples were taken at min 5', 10', 20', 30', 60', 90', 120', 180', and 240', then immediately transferred to two series of plastic tubes, containing either Na-EDTA (6% w/vol), for glucose, amino acid, insulin and C-peptide determinations, or a protease inhibitor (EMD Millipore Corporation, Merck KGaA, Darmstadt, Germany), for GIP and GLP-1 assay [...]

Key References:

1. Nilsson M, Stenberg M, Frid AH, Holst JJ, and Björck IME. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins. *Am J Clin Nutr* 2004;80:1246–53.
2. Hartmann R and Meisel H. Food-derived peptides with biological activity: from research to food applications. *Current Opinion in Biotechnology* 2007, 18:163–169.
3. Tessari P, Kiwanuka E, Cristini M, Zaramella M, Enslin M, Zurlo C, Garcia-Rodenas C. Slow versus fast proteins in the stimulation of beta-cell response and the activation of the entero-insular axis in type 2 diabetes. *Diabetes Metab Res Rev*. 2007 Jul;23(5):378-85
4. Gunnerud U, Holst JJ, Östman E, and Björck I. The glycaemic, insulinemic and plasma amino acid responses to equi-carbohydrate milk meals, a pilot- study of bovine and human milk. *Nutrition Journal* 2012, 11:83.
5. Marshall K, Therapeutic Applications of Whey Protein, *Altern Med Rev* 2004;9(2):136-156.