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^{45}Ca as a marker to assess portal absorption of calcium from milk in the growing pig

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Abstract – The aim of this experiment was to determine portal absorption of calcium in the growing pig after ingestion of extrinsic labelled ^{45}Ca milk. Portal levels of ^{45}Ca are high very soon (15 min) after ingestion of milk and decrease after 60 min. The absorption peak was observed at 30 min. The highest porto-arterial differences were observed between 15 and 60 min, indicating that absorption mainly occurs during the first hour (about 48%). ^{45}Ca portal absorption rate is close to 76%; it indicates that the major part of the calcium is absorbed in the first four postprandial hours.

Absorption / calcium / milk / pig

Résumé – **Utilisation du ^{45}Ca comme marqueur pour évaluer l'absorption portale du calcium du lait chez le porc en croissance.** Le but de cette étude a été d'étudier l'absorption portale du calcium chez le porc en croissance après l'ingestion du lait marqué extrinsèquement par l'isotope ^{45}Ca. L'apparition portale du ^{45}Ca est très rapide (15 min) et le pic d'absorption est situé 30 min après l'ingestion du lait. Les différences porto-artérielles les plus élevées sont observées entre 15 et 60 min, indiquant que l'absorption se produit principalement pendant la première heure (48 %). Le taux d'absorption du ^{45}Ca (environ 76 %) indique que la majeure partie du calcium est absorbée dans les quatre premières heures postprandiales.

Absorption / calcium / lait / porc

1. INTRODUCTION

The most convenient source of calcium in terms of cost and accessibility is milk and dairy products which constitute thus a major source of dietary calcium [4, 25]. There is no standard way of measuring calcium absorption. Different methods exist [1, 8, 10, 28], ranging from intubation with a lumen tube to calcium balance procedures and portal absorption methods. To determine absorption of dietary calcium, it is

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necessary to distinguish it from endogenous calcium [1]. The use of labelled milk has made it possible to specify calcium bioavailability in milk using balance methods or measurements of labelled calcium in peripheral blood [2, 9, 24, 29]. ^{45}Ca has been considered as a valuable marker for nutritional studies [24] and it is known that ^{45}Ca added to milk is highly incorporated both in the aqueous phase and in the colloïdal phase [11].

The nutritive value of dietary calcium is linked to subsequent postprandial calcium availability in the portal blood. To our knowledge no information is available on portal absorption of calcium after ingestion of milk. A better knowledge of calcium appearance in the portal blood should enable an estimation of the calcium bioavailability to the organism. Portal absorption of nutrients cannot be studied in vivo in humans, but pigs provide a valid model to conduct such studies. Indeed, the pig seems to be a good model for humans for studying the calcium phosphorus metabolism [12].

Thus, the aim of this study was to establish the porto-arterial kinetics of ^{45}Ca and total calcium and to estimate the ^{45}Ca portal absorption from extrinsic labelled milk in the growing pig.

2. MATERIALS AND METHODS

2.1. Preparation of ^{45}Ca labelled milk

400 mL of milk was spiked with 30 μCi (1080 kBq) of ^{45}Ca (31 μg ^{45}Ca·mCi^{-1}, ChemSyn laboratories, Lenexa, Kansas, USA). The labelled milk was carefully mechanically mixed to ensure a good distribution of the ^{45}Ca both in the aqueous phase and in the colloidal phase. After mixing, the labelled milk was stored for 60 min at ambient temperature before ingestion by the animal.

2.2. Animals and diets

The animal protocol was in accordance with the general guidelines of the Council of European Communities (1986) [3] for the care and use of laboratory animals. Four castrated male Large White pigs (body weight 43–47 kg) from the herd of a commercial farm (EARL BIMA, 54160 Pulligny, France) were used. For one week before the experiment the animals were kept in the facilities of our laboratory and received a well-balanced diet (800 g meal) based on wheat and soybean to ensure the maintenance and growing needs of the animals [7]. Each animal was fitted with two catheters, one placed in the portal vein and one in the brachiocephalic artery [15, 21]. Anaesthesia was induced with sodium thiopentane (10 to 15 mg·kg^{-1}) and maintained with fluothane inhalation (0.5 to 1.5% as required). The animals were intubated with a cuffed endotracheal tube and the lungs were mechanically ventilated at a minute volume of 150 mL·kg^{-1}. Surgery was performed under very strict aseptic conditions. The animals began to eat the day after the operation and rapidly recovered their normal growth rate (400 g·d^{-1}). To prevent obstruction by blood clots the cannulaes were rinsed daily with a heparinised (100 IU·mL^{-1}) NaCl solution (9 g·L^{-1}). This was done under aseptic conditions to avoid any risk of infection. The experimental period began when the pigs had completely recovered from surgery (5–6 d). Throughout the experimental period, they were kept in individual cages allowing easy access to the cannulae.

2.3. Experimental measurements

At 10, 15 and 20 d postsurgery, 400 mL milk were given orally to the animals after a fasting period of 12 h. For each experimental day, portal and arterial blood samples (5 mL) were collected simultaneously prior to the milk supply and at 15, 30, 60, 90, 120, 150, 180, 210 and 240 min after ingestion.

Blood was replaced by equal amounts of sterile heparinised NaCl (9 g·L^{-1}) solution. The packed cell volume ranged from 27–34% without any apparent influence of the sampling on the relative part of plasma in whole blood. Blood samples were immediately centrifuged for 10 min at 3000 *g* (4 °C). Plasma supernatant was then collected and stored at –20 °C. ^{45}Ca in plasma was measured by direct counting (10 min) of duplicate 1 mL samples in 10 mL Ultimagold scintillation fluid (Beckman, 93220 Gagny, France) using a Tricarb 460 CD liquid scintillation counter (Packard, 94533 Rungis, France). Radioactivity is expressed in Bq per mL of plasma. Total calcium was analysed by synchrom® Cx systems (Calcium reagent, Beckman Counter, 93220 Gagny, France). ^{45}Ca and total calcium concentration in portal and arterial blood from day 10, 15 and 20 were averaged per animal in order to get valuable and precise portal and arterial kinetics data.

2.4. Portal absorption calculations

Postprandial kinetics of total calcium and ^{45}Ca in the portal vein and the arterial blood was determined as well as postprandial kinetics of porto-arterial concentration differences (PAD). Portal absorption of ^{45}Ca was calculated as: "^{45}Ca porto-arterial differences × blood flow". ^{45}Ca meal absorption rate was calculated as: "portal absorption of ^{45}Ca / ^{45}Ca content in the meal". Blood flow per min and per kg body weight could be estimated thanks to many references using growing pigs [5, 6, 14, 16–19, 21, 26]. In fact, it is known that meal ingestion is followed by a small rise in portal blood flow during the first 1–2 postprandial hours and individual variations in pig portal blood flow have been established at between 2.8 and 5.7% [20]. Several authors found relatively constant blood flow values after ingestion of the meal [5, 6]. One can easily assume that portal blood flow variations in the present work were similar for all the animals. In this study, we have calculated portal absorption with a constant blood flow value of 41 mL·min^{-1}·kg^{-1} body weight, which corresponds to the mean value from observations of different authors (Tab. I) and with mean ^{45}Ca PAD values for milk for the 0–240 min studied time period.

Table I. Blood flow references in the growing pig.

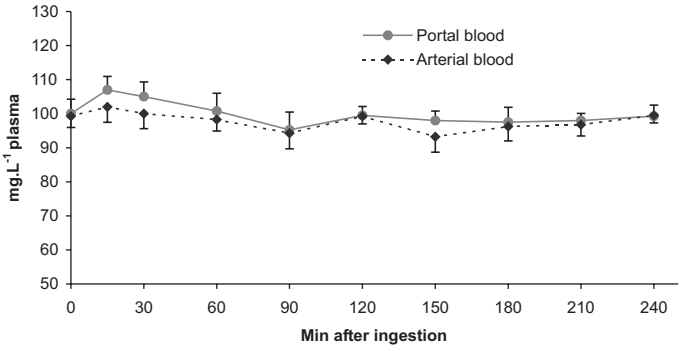
Body weight (kg)	Blood flow (mL·min^{-1}·kg^{-1})	Authors
65	37.6	[5]
61	39.7	[6]
8	44.7	[14]
45	41	[16]
45	37.9	[17]
40	49.9	[18]
22	45.2	[19]
64	42	[21]
57	32.8	[26]
mean value: 41.2		

2.5. Statistical analysis

Statistical analysis [23] involved calculation of the mean and standard error. The Student's t-test was used for comparison of the means at a significant level of 0.05. Data are presented as mean + standard error.

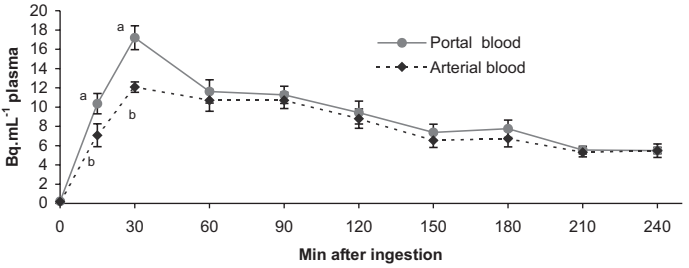
3. RESULTS

Figures 1 and 2 indicate postprandial portal and arterial kinetics of total calcium and ^{45}Ca after ingestion of 400 mL of extrinsic labelled milk. Portal and arterial total calcium concentrations were quite similar during the studied period (Fig. 1). No significant differences were found



No significant differences whatever the time point ($P < 0.05$)

Figure 1. Portal and arterial kinetics of total calcium after ingestion of 400 mL labelled milk in the growing pig (mean ± standard error, n = 4).



(a, b): for each time point, means without a common letter are significantly different ($P < 0.05$).

Figure 2. Porto-arterial kinetics of ^{45}Ca after ingestion of 400 mL labelled milk in the growing pig (mean ± standard error, n = 4).

between portal and arterial concentration throughout the studied period (0–240 min after ingestion). Thus, kinetics of total calcium do not enable a precise description of the milk calcium absorption profile and reveal the need to use a marker to specify the absorptive behaviour of food calcium. Portal and arterial levels of ^{45}Ca increase very soon after the oral ingestion of milk and decrease after 60 min, indicating very fast intestinal transit and absorption of milk calcium (Fig. 2). The absorption peak appears at 30 min. Two main steps in the absorption profile of ^{45}Ca can be distinguished: the first 60 min following ingestion and the later period after 60 min. The highest porto-arterial differences were observed in the period between 15 min and 60 min (Fig. 2). At 15 and 30 min after ingestion, portal ^{45}Ca levels are significantly higher than arterial ^{45}Ca levels.

According to our calculations, ^{45}Ca portal absorption shows that the main absorption occurs during the first hour (about 48%) (Tab. II). During the 0–240 min time period, the ^{45}Ca absorption rate is close to 76% (Tab. II).

Table II. Portal absorption of ^{45}Ca after oral ingestion of 400 mL extrinsic labelled (1080 kBq) milk in the growing pig. (n = 4)

Absorption period (H)	kBq	% ingested dose
1	522.6	48.39
2	104.7	9.70
3	162.7	15.07
4	36.4	3.37
0–4 h	826.5	76.53

4. DISCUSSION

The aim of this investigation was to study total calcium and ^{45}Ca portal absorption after ingestion of 400 mL extrinsic labelled milk. The extrinsic labelling approach has been used in many studies of calcium bioavailability [2, 9, 13, 27] and for several authors who compared extrinsic and intrinsic labelling methods, the fractional absorption of calcium from milk was not affected by the method of labelling [8, 9]. The measuring of these events is of great physiological importance since it enables the determination of the specific absorption profile of dietary calcium and contribution of milk to calcium absorption.

Total calcium kinetics (Fig. 1) are difficult to interpret because it is not possible at this stage to distinguish calcium which has been brought by the dairy products from endogenous calcium including bone metabolism [1]. Thus, isotopic tracer methods appear necessary to evaluate calcium bioavailability from dairy foods. ^{45}Ca appears to be a valuable marker since it allows the establishment of the specific absorption profile of calcium from milk.

^{45}Ca portal and arterial kinetics (Fig. 2) indicate for the first time the specific absorption profile of milk calcium. The postprandial portal and arterial kinetics of ^{45}Ca were already high 15 min after the start of milk ingestion (Fig. 2). These results can be related to previous observations [22]. These authors observed a net ^{45}Ca blood peripheral appearance as soon as 30 min after feeding milk to men. The greatest portal and arterial differences in values were found between 15 min and 60 min (Fig. 1) indicating that ^{45}Ca is highly absorbed within the 0–60 min time period. Maximun absorption is observed at 30 min.

These results indicate that around 76% of ^{45}Ca from milk is absorbed by the organism. Since the amount of ^{45}Ca added to the milk was quite low (about 1 μg) compared to total milk calcium (about 500 mg in 400 mL) and was carefully mixed into the milk, we can expect, in agreement with previous authors [2, 9], that ^{45}Ca absorption is representative of total milk calcium. Thus, our data (Fig. 2, Tab. II) demonstrate that milk calcium is highly absorbed as was suggested by several authors who also used ^{45}Ca methods to study calcium bioavailability of milk products [2, 9, 13, 27].

These results demonstrate a new and interesting way to assess bioavailability of calcium from milk. It would now be of great interest to perform further experiments to compare calcium bioavailability from different calcium sources such as milk products or water.

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