Use of magnetic resonance imaging in the study of goat mammary glands *in vivo**

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Summary. A technique is described for the in-vivo determination of mammary gland size and gross composition in goats by using nuclear magnetic resonance imaging (MRI). The volume of test objects determined with MRI had an error of $+0.4 \pm 1.6\%$ of the actual volume. In lactating goats the in-vivo MRI estimate of mammary parenchymal volume was significantly greater than, but highly significantly correlated with, the weight of parenchyma determined *post mortem* (for the whole udder, $r = 0.88$, $P < 0.001$; for individual glands, $r = 0.85$, $P < 0.001$). MRI-determined estimates of the volume of fluid within the mammary gland were within 1.2% of the volume of milk removed from the udders after imaging. The spin-lattice (T_1) relaxation time of the whole udder correlated closely with the volume of fluid within the udder. The T_1 relaxation time of parenchymal tissue measured *in vivo* did not differ significantly from that determined immediately after post-mortem excision.

Keywords: mammary gland; magnetic resonance imaging; methodology; goat; lactation

Introduction

Mammary development and function have been intensively investigated in laboratory species, but parallel studies in dairy animals, including goats, have been hampered by the lack of a suitably non-invasive technique for monitoring secretory tissue mass *in vivo*. Available methodologies such as water displacement and plaster casts (Linzell, 1966) determine only the gross external dimensions, and take no account of variation in internal composition. A well developed udder consisting of 80% secretory tissue and 20% stroma would be indistinguishable from another of the same size but containing 20% parenchyma and 80% stroma. For the same reason, water displacement cannot measure individual glands independently, when two or more are tightly juxtaposed, as in the udder of the goat.

Whole-body imaging techniques such as magnetic resonance imaging (MRI) and X-ray computed tomography (X-ray CT) have the potential to provide information about gross internal composition of the gland, as well as its overall size. The ability of X-ray CT to discriminate between fatty stroma and ductal parenchyma has been demonstrated in heifers (Sejrsen *et al.*, 1986; Sørensen *et al.*, 1987), although these measurements were made *in vitro* on excised udders, since heifers are too big for conventional imagers. Preliminary studies suggested that goats could be studied *in vivo* using MRI (Foster & Knight, 1983).

In MRI the hydrogen nuclei of water and lipid molecules are excited by electromagnetic radiation in the presence of a magnetic field, resulting in a detectable signal. The magnitude of the signal and the subsequent relaxation to the pre-excited state are measured. The use of magnetic field gradients allows this information to be collected from spatially defined parts of the body.

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Water content varies between tissues and the water molecules have constraints upon their motion arising from hydration layers around larger molecules. The amount of water and lipid, and their freedom of motion, define signal size and NMR relaxation characteristics, such as the spin-lattice relaxation time (T_1). This permits discrimination between tissues and the production of interpretable images of slices through the body (Hutchison *et al.*, 1980; Foster & Hutchison, 1985).

The aim of the present study was to determine the potential uses of MRI in the study of mammary tissue *in vivo*.

Materials and Methods

Animals. Lactating British Saanen goats, housed at the Rowett Research Institute, Aberdeen, were milked twice daily at approximately 08:00 and 16:00 h. They were fed a concentrate diet (500–1500 g/day depending on stage of lactation) with hay and water available *ad libitum*.

The NMR imager. The Aberdeen Mk I NMR imager operates at a magnetic field strength of 0.04 T (proton frequency of 1.7 MHz), and has a bore diameter of 49 cm, which limits the size of goat that can be imaged to under 100 kg. An interleaved saturation recovery/inversion recovery pulse sequence with a repetition time (T_r) of 1000 msec and an interval (T_i) of 200 msec was used. Transaxial slices 12 mm thick were collected through the body at 2-cm intervals. From the data of each 4.3-min collection 2 fundamental image types were derived. Proton density (PD) images, which give information about the total proton spin populations (the amount of water and fat protons), were derived from the saturation recovery (SR) signal, while inversion recovery (IR) images, which contain PD information modified by the T_1 relaxation time, were derived from the IR signal. From these 2 basic image types the spin-lattice (T_1) relaxation time image was calculated. Other arithmetic combinations of the basic images are possible and one used in the present study was the difference image (D: PD – IR). On the Aberdeen system each image is displayed within 30 sec of the end of each collection period, enabling decisions to be made concerning the positioning of the next image. Images were collected as 128×128 pixel grids, each pixel being a square equivalent to 0.352×0.352 cm. Therefore structures smaller than 5 mm were not resolvable.

Standard method for goat imaging. Before imaging, each goat was hand milked with the aid of exogenous oxytocin (200 mU i.v.) and sedated using xylazine hydrochloride (Rompun; Bayer, Bury St Edmunds, Suffolk, UK), 0.012 ml/kg i.v. The goat was placed on its left side on a sliding couch marked with a centimetre scale, and pushed into the imager. The posterior end of the udder was located using the couch scale and an image collected at that point. Images were then collected at 4-cm intervals by moving the goat in a posterior direction after each image. Once the anterior end of the udder was passed, images were staggered by 2 cm and then collected at 4-cm intervals in the opposite direction. This process was continued until the entire anterior–posterior extent of the udder had been imaged at 2-cm intervals.

Image analysis. Images were stored on movable magnetic hard discs with a capacity of approximately 12 000 images. Analysis of the images was carried out using a MicroVax II minicomputer and image display hardware (advanced raster graphics system) running in-house image display software. During image analysis PD, D, IR and T_1 images were displayed simultaneously (Fig. 1). The edge of the udder, mammary gland, or test object was outlined on the D image semi-automatically by the computer, and displayed on all 4 images. This process involves setting a threshold specific to mammary tissue or test object, the computer then fills all areas above the threshold. The computer-selected area is then 'trimmed' interactively, using a mouse which controls a display screen cursor, to conform to the outline of the mammary tissue or test object. Refinements, such as separating the 2 glands of the udder, were also carried out interactively. Fat surrounding the mammary gland was excluded interactively using the mammary tissue outline derived from the D image but displayed on the IR image. Blood vessels associated with mammary tissue were also excluded. Figure 2 shows an IR image with the different areas of mammary tissue outlined. Statistics from any particular region of interest, such as mean area (cm^2) and mean T_1 relaxation time (msec), were displayed on the user terminal.

The volumes (v) of the udders and test objects were calculated from serial transaxial images using slice areas (a_1 , a_2) from consecutive pairs of images with a separation (d) between slices, by means of a simple truncated cone model (Kvist *et al.*, 1986):

$$v = \Sigma d/3[a_1 + a_2] + (\sqrt{[a_1 \cdot a_2]})$$

Accuracy of the method. Containers of simple or complex shape (plastic bottles, disposable surgical gloves) and 300–4500 ml capacity were filled with dilute CuSO_4 solution, to simulate the T_1 characteristics of mammary tissues, and imaged with slice intervals of 2 cm. An excised goat udder was imaged, also with a slice interval of 2 cm. The volume of each of these test objects was calculated from the images as described above (i.e. using the D image) and then the calculation was repeated using each of the other 3 image types as the basis for deriving the cross-sectional information. The calculated volumes were compared with known volumes and weights. The complete analysis was performed 4 times to assess reproducibility.

Fig. 1. An example of the 4 MRI image types used. All 4 images are versions of the same slice through a goat lying on its left side with only the right mammary gland milked-out before imaging: (a) PD image, (b) D image, (c) IR image, (d) T_1 relaxation time image: (e) labelled drawing illustrating the main features visible.

Using the standard procedure described, the volumes of the whole udder and each gland independently were calculated for 17 goats. Stage of lactation ranged from 15 to 30 weeks. Within 12 days after imaging, the goats were killed and the udders quickly excised, weighed (gross weight) and chilled on ice. All the skin, teat, gland cistern, major ducts, blood vessels and dissectable fat were trimmed away from the parenchyma, which was then weighed (parenchyma weight). The calculated volume (MRI) was compared with the gross and parenchyma weights, for the whole udder and also for each individual gland. In 10 of the goats udder volume was measured by water displacement 24 h before death, for comparison with MRI and in-vitro measurements.

Five goats were imaged twice on consecutive days. On the first occasion the standard procedure was used, but on the 2nd day milking was delayed until after imaging, whereupon the volume of milk removed was recorded. The two sets of images were thus of 'empty' and 'full' udders. During image analysis, fluid within the udder was identified and an estimate made of its volume; the difference in fluid volumes between the empty and full udders was then compared with the volume of milk removed at milking.

Fig. 2. An IR image through a goat lying on its left side. Both the milked (M) and un milked (UM) glands, and the fluid within the un milked gland (C) are shown outlined.

T_1 characteristics. Measurements of the T_1 value of mammary parenchyma *in vivo* were compared with *in-vitro* determinations made within 4 h of death in 5 goats. The *in-vitro* measurements were made on small (0.5–1 g) samples of excised, blotted parenchyma in a home-built NMR relaxometer operating at 2.5 MHz and 37°C. A Hahn spin-echo pulse sequence with prior spin inversion was used with 15 different tau values to provide the relaxation curve. This was analysed assuming a single exponential decay by a three-point least-squares fit method. Each T_1 relaxation value was the mean of 5 repeated measurements.

The effect of retained fluid on parenchyma T_1 was assessed *in vivo* in 4 goats. They were imaged twice on consecutive days, once after milking with oxytocin and once after milking by intraductal catheter (Henderson & Peaker, 1987), which removes cisternal but not alveolar milk.

Statistical analysis. Statistical analysis was by analysis of variance (ANOVA) or paired *t* test, as appropriate. Relationships between variables were tested by least-squared linear regression analysis, and correlation coefficients were determined. Values are presented as mean $\pm$ s.e.m.

Results

Test objects

Errors in volume calculation were determined by subtracting the calculated from the known volume and expressing the result as a percentage of the latter. Taking all test objects together, error varied from $-4.3 \pm 2.7\%$ to $+3.5 \pm 2.9\%$, depending on which image type was used, although none of the determinations was significantly different from each other or from the actual volume of the test object. The error obtained with simple shapes ($+0.3 \pm 4.2\%$) was not significantly less than that obtained with complex ones ($-3.3 \pm 5.0\%$ for the excised udder). Increasing the slice interval from 2 cm to 4 cm increased the error non-significantly ($-2.7 \pm 0.9\%$ to $-5.1 \pm 4.7\%$). Repeated determinations on the same test object yielded a range of errors between $-2.1 \pm 6.4\%$ and $+0.1 \pm 2.6\%$.

There was a linear and proportional relationship between MRI-calculated and known volumes of test objects, within the range 300–4500 ml (Fig. 3). Least-squares linear regression of MRI calculated on known volumes yielded a slope of 1 with an intercept offset of 10.7 ml. The total error of volume calculation was $+0.4 \pm 1.6\%$ using all 4 MRI image types.

Fig. 3. The relationship between MRI-calculated and measured volumes of test objects.

In-vivo volume determinations

Using the standard method, imaging time was between 50 and 65 min/goat, depending upon the size of the udder. Occasionally (4% of imaging sessions) sedation was of insufficient duration to allow complete collection of all 2 cm slices, and then volume calculation was based on 4 cm slices. The udder of the goat was most clearly delineated in the D image. Discrimination between parenchyma and fatty stroma was best achieved in the IR image, and the T_1 image was used for identifying fluid within the udder. Figures 1 and 2 show the udder of a lactating goat with one un milked gland and one milked gland. The whole udder, the individual glands and the fluid present in the un milked gland have been outlined in Fig. 2.

The estimate of mammary volume produced by MRI for the 17 lactating goats was 1696 ± 100 ml; the measured gross weight was 1892 ± 116 g, and the parenchyma weight was 1103 ± 101 g. Overall, MRI underestimated gross weight by 10.4% and overestimated parenchyma weight by 53.8%. Linear regression analysis demonstrated highly significant correlations between MRI estimates and both gross and parenchyma weights:

$$\text{MRI estimate} = 294 + 0.741 \times \text{gross weight} \quad (r = 0.87, P < 0.001, R^2 = 75.0\%)$$

$$\text{MRI estimate} = 735 + 0.871 \times \text{parenchyma weight} \quad (r = 0.88, P < 0.001, R^2 = 76.1\%)$$

The parenchyma weight relationship is shown in Fig. 4.

Udder volume was determined by water displacement in 10 goats. Correlations with parenchyma weight were 0.86 ($P < 0.001$) for MRI and 0.83, ($P < 0.001$) for water displacement. These data were heavily influenced by the exceptionally large udder of one goat. Excluding this datum point resulted in correlations of 0.80 ($P < 0.001$, MRI) and 0.09 (n.s., water displacement). The parenchymal portion of the udder ranged from 48% to 69% of gross mammary weight in the 10 goats. Ranking into 2 groups of 'highest' ($65.5 \pm 1.3\%$) and 'lowest' ($53.6 \pm 1.7\%$) parenchymal content produced groups with significantly different parenchymal mass (1496 ± 221 versus 965 ± 64 g, $P < 0.05$) and MRI estimated volumes (2077 ± 185 versus 1404 ± 141 ml, $P < 0.05$), but similar udder volumes by water displacement (2049 ± 266 versus 1837 ± 101 , n.s.).

Estimates of individual gland size by MRI were also highly correlated with both the gross weight ($r = 0.91$, $P < 0.001$) and the weight of parenchyma ($r = 0.85$, $P < 0.001$) of the individual

Fig. 4. The relationship between the MRI estimate of parenchyma volume and the actual weight of parenchyma determined *post mortem* for a group of 17 goats.

glands. In 15 of 17 goats MRI correctly identified the larger of the two glands. Overall, the ratio between the right and left glands was 0.92 ± 0.06 by parenchymal weight and 0.97 ± 0.05 by MRI; the two determinations were not significantly different from each other.

The volume of fluid retained in the udders of 4 goats was 103 ± 48 ml when they were milked before imaging (empty udder) and 623 ± 128 ml when they were milked after imaging (full udder), a difference of 521 ± 102 ml. This estimate of removable milk agreed almost exactly with the volume of milk subsequently removed from the full glands (515 ± 101 ml).

T₁ characteristics

In-vivo determinations of T₁ relaxation time from regions consisting primarily of parenchyma were not significantly different from in-vitro measurements of parenchyma T₁ (269 ± 18 versus 263 ± 29 msec). The overall T₁ value for the whole udder was considerably greater for unmilked glands than for those which had been milked (449 ± 37 versus 272 ± 10 msec, $P < 0.001$, *t* test). Prior milking with oxytocin reduced the in-vivo parenchyma T₁ estimate compared to that obtained when the gland cistern was emptied by catheter but alveolar milk was retained (260 ± 3 versus 287 ± 7 msec, $P < 0.05$, *t* test). The correlation between whole-udder T₁ and fluid content of the gland was high ($r = 0.98$, $P < 0.001$).

Discussion

Previous studies of mammary development have been limited to external examination, post-mortem analysis or biopsy (Knight, 1984). Measurement of udder volume by Archimedes' principle of water displacement (Linzell, 1966) is useful for comparing udders of relatively similar composition, but the present results demonstrate that parenchymal content does vary between goats (even during lactation) and erroneous conclusions can result. Plaster casts (Ingelby, 1949) and wire-mesh modelling (Junge, 1963) suffer from the same limitation, i.e. they determine only gross volume. An imaging technique is required if the relative content and absolute amount of parenchyma is to be determined *in vivo*.

Sørensen *et al.* (1987) explored the use of X-ray CT for quantitative analysis of the excised udders of heifers, which consist of relatively small amounts of ductal parenchyma lying within a large fat pad, i.e. the parenchyma content is small and rather variable. The CT estimate of parenchyma volume was considerably lower than the weight of dissected parenchyma, but very similar to the calculated weight of 'true' parenchyma, based on histological analysis of the dissected tissue. It was concluded that CT excluded extra-parenchymal tissue (chiefly fat) more reliably than did physical dissection. CT has not been used to study mammary tissue *in vivo*, but has been used for *in-vivo* whole-body analysis of goats, pigs and sheep (see Foster *et al.*, 1988, for review).

We have previously reported preliminary data obtained with MRI on mammaryogenesis in goats (Foster & Knight, 1983; Fowler *et al.*, 1988). The present results indicate that the straightforward determination of volume using MRI is accurate and reproducible; test objects were measured with an error of 1–2%. Estimation of mammary parenchyma *in vivo* is less precise, because of the additional requirement of first differentiating between parenchyma, mammary stroma and the rest of the body. The fact that the MRI estimate fell between the gross weight and the parenchyma weight indicates that some but not all of the extraparenchymal tissue was excluded from the calculation. The excellent correlation between the MRI estimate of parenchyma volume and the actual weight of parenchyma suggests that the method is reliable. Most importantly, MRI correctly predicted differences in the absolute amount of parenchyma which were due to variation in the composition of the udder. These compositional differences were small in comparison to the changes which occur during the gestational development of the gland, and so it is highly probable that MRI would correctly identify these latter changes as well.

In the present study, structures smaller than 5 mm were not resolvable in the images. This places the median suspensory ligament which divides the two mammary glands of the goat at the very limit of resolution. Nevertheless, visual discrimination between the two individual glands was possible in the majority of images, and by cross-referring between adjacent images (16 images can be displayed simultaneously) it was possible to identify reliably the mid-line in all images. MRI is therefore able to measure individual glands *in vivo*, a vital consideration in experiments investigating local control mechanisms, in which treatment is applied unilaterally to one gland only.

The relationship between intramammary fluid and mammary T_1 was expected, given that tissue water affects T_1 relaxation rates (Foster & Hutchison, 1985). Thus whole-udder T_1 was greater in unmilked glands than milked ones, and parenchyma T_1 was reduced by the milk-ejecting effect of oxytocin.

MRI is essentially a clinical diagnostic tool, and has been used for a variety of applications, including study of the human breast (El Yousef *et al.*, 1984). Foster *et al.* (1988) have reviewed potential research applications of MRI in the area of whole-body compositional analysis. The present results indicate that the technique will also be useful for studying the development of individual tissues, in this case the mammary gland. It enables accurate estimates of parenchyma volume to be made repeatedly in individual animals, thus allowing serial study through the course of reproductive cycles or treatment periods.

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