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# Effect of monosaccharide sugars on LH-induced differentiation and sugar transport facilitator (SLC2A) expression in sheep theca cells *in vitro*

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**Abstract.** The aim of the present study was to investigate the effects of glucose, galactose and fructose on the LH-induced differentiation and mRNA expression of sugar transport facilitators (*SLC2A*) by sheep thecal cells derived from small antral follicles cultured under serum-free conditions for 6 days. The dose and type of monosaccharide had a significant effect on LH-induced androstenedione production by theca cells and there was a significant interaction ($P < 0.001$). Glucose and galactose were used with equal efficiency so that cell numbers and androstenedione production at the end of the culture were comparable. Pharmacological doses of glucose (16.7 mM) inhibited steroidogenesis ($P < 0.05$). Cell numbers and androstenedione production by cells cultured with fructose were lower than for cells cultured with either glucose or galactose ($P < 0.001$). None of the monosaccharides resulted in the production of lactate. Expression of *SLC2A1*, *SLC2A4* and *SLC2A8*, but not *SLC2A5*, mRNA was detected in fresh and cultured theca cells. Large doses (16.7 mM) of glucose and fructose, but not galactose, suppressed ($P < 0.05$) *SLC2A* expression. The results show that glucose and galactose, but not fructose, are readily metabolised via oxidative pathways to support LH-induced differentiation of sheep theca cells. Further work is required to determine the mechanisms resulting in these differences in relation to the established effects of nutrition on reproductive function.

**Additional keywords:** fructose, galactose, glucose.

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## Introduction

In mammalian species, ovarian function can be influenced by environmental factors, such as nutrition, but the underlying mechanisms remain obscure (Scaramuzzi *et al.* 2006). The catabolism of glucose to produce ATP is the primary metabolic pathway to support cellular processes, and there is abundant evidence that this is also the case in the ovary. Evidence of glucose transporter expression (SLC2A1 and SLC2A4) on granulosa and theca cells of sheep antral follicles (Williams *et al.* 2001) and ovarian arteriovenous difference studies suggest that glucose is taken up by the ovary in significant amounts (Rabiee *et al.* 1997; Scaramuzzi *et al.* 2010). Furthermore, studies using cultured cells in our laboratory have shown that glucose, metabolised under anoxic conditions to lactate, is the preferred energy substrate to support the gonadotrophin-induced differentiation of ovine granulosa cells (Campbell *et al.* 2010*b*).

In addition to glucose, many foods contain other monosaccharides that may also mediate nutritional effects on follicular function. Fructose, the predominant blood sugar in fetal ruminants (Daniels *et al.* 1974), is also an important energy substrate for the testis (Burant *et al.* 1992) and is a physiologically significant monosaccharide in human nutrition because it occurs in high levels in fruit and corn syrup (Elliott *et al.* 2002). SLC2A5 (Glut5), the fructose-specific member of the solute carrier family, is highly expressed in testes and the small intestine (Burant *et al.* 1992). In the testis, fructose is used by mature spermatids, having been taken up from the seminal fluid, and therefore represents a major source of metabolic energy (Burant *et al.* 1992). Conversely, the evidence that fructose has a role in ovarian physiology is equivocal. Although expression of SLC2A5 has not been detected in rat ovary (Kol *et al.* 1997) or ovine granulosa cells (Campbell *et al.* 2010*b*), fructose can

support the FSH-induced differentiation of cultured granulosa cells (Campbell *et al.* 2010*b*). In primates, including humans, fructose does not affect leptin or insulin release or blood glucose levels (Curry 1989; Havel 1997; Teff *et al.* 2004). However, in ruminants fructose is rapidly metabolised to glucose in the liver (Luick *et al.* 1957), which may then trigger insulin release. We have observed increased insulin secretion in response to challenges with fructose in sheep that differs in terms of duration and magnitude to identical challenges with glucose (Campbell *et al.* 2010*a*). Furthermore, both direct ovarian and systemic infusions of fructose have a stimulatory effect on ovarian oestradiol and androstenedione secretion (Campbell *et al.* 2010*a*).

The expression of several SLC2As that transport galactose (SLCA21, SLCA22, SLCA23 and SLCA28) has been reported in the ruminant ovary (Williams *et al.* 2001; Nishimoto *et al.* 2006; Pisani *et al.* 2008; Campbell *et al.* 2010*b*). Furthermore, rodent and human ovaries express high levels of the enzymes that metabolise galactose via the Leloir pathway (Liu *et al.* 2000), and accumulation of galactose or its metabolites in humans results in infertility due to impaired ovarian function. This condition typically occurs in women with a deficiency in or a mutation on one of the key enzymes required for galactose metabolism, namely galactose-1-phosphate uridyltransferase (GALT). Little is known of the patterns of expression of these enzymes in ruminants, but these toxic effects of galactose are consistent with the observation that high doses of galactose inhibit the FSH-induced differentiation of sheep granulosa cells (Campbell *et al.* 2010*b*) and ovarian oestradiol and androstenedione secretion following direct ovarian infusion in sheep (Campbell *et al.* 2010*a*). Despite a theoretical availability of the glycolytic pathway for galactose metabolism, we have found that of the monosaccharide tested, galactose was the least well used by ovine granulosa cells, with very little lactate being synthesised compared with glucose or fructose (Campbell *et al.* 2010*b*). Further elucidation of the role of galactose on ovarian function in the ruminant is therefore a research priority.

To summarise, there is abundant evidence that monosaccharides, such as fructose and galactose in addition to glucose, could be key mediators of the effects of nutrition on ovarian function. To test the hypothesis that different monosaccharides will be used differentially by ovarian theca cells, we investigated the effect of the dose and type of monosaccharide on LH-induced differentiation and the expression of sugar transport facilitators in ovine theca cells in culture. The primary marker of LH-induced differentiation used was androstenedione production because the serum-free theca cell culture system used in the present study has been specifically designed to allow gonadotrophic induction of enhanced steroidogenesis in cells derived from small antral gonadotrophin-responsive follicles in a time frame that parallels the recruitment of follicles into the gonadotrophin-dependent cohort *in vivo* (Campbell *et al.* 1998).

## Materials and methods

### *Theca cell culture*

Unless stated otherwise, all reagents were purchased from Sigma-Aldrich (Poole, Dorset, UK). Theca cells were isolated and cultured using the procedures described previously for ovine tissue (Campbell *et al.* 1998). Briefly, small (1–3 mm diameter) antral follicles were isolated from ovaries collected from non-pregnant animals at a commercial abattoir. This size range of follicles was used because it encompasses the recruitable cohort of gonadotrophin-responsive follicles in this species (McNeilly *et al.* 1991) and, as detailed above, the culture system is specifically designed to allow gonadotrophin-induced differentiation of the theca cells from these gonadotrophin-responsive follicles in response to physiological doses of LH over a 4–6-day period (Campbell *et al.* 1998). In addition, because this cohort of gonadotrophin-responsive follicles continues to develop even under hypogonadotrophic conditions (McNeilly *et al.* 1991), these follicles are relatively non-responsive to differences in changes in the levels of pituitary gonadotrophins associated with different reproductive states, such as stages of the breeding season or stages of the oestrus cycle. Therefore, this abattoir-derived tissue is both a physiologically and ethically acceptable source of tissue for these *in vitro* studies.

Follicle dissection was performed in Medium 199 supplemented with HEPES, antibiotics and antimycotics. Follicles were hemisected in Dulbecco's phosphate-buffered saline without calcium or magnesium ($DPBS^-$) and the follicle halves were flushed repeatedly with a 1-mL syringe. The theca shells were allowed to settle before being transferred to a fresh container for dispersion by incubation in an enzyme mix containing 5 mg $mL^{-1}$ collagenase, 1 mg $mL^{-1}$ hyaluronidase, 1 mg $mL^{-1}$ protease and 0.002% donor calf serum (DCS; v/v) for 30–45 min at 37°C. After 20 min incubation, 200 μg deoxyribonuclease was added. Incubation was continued until the theca cells were completely dispersed and the reaction was then stopped by the addition of 2 mL DCS. After centrifugation at room temperature for 10 min at 800 rpm, the cell pellets were resuspended in culture medium and, after washing with culture media, the number and viability of cells was determined by Trypan blue exclusion. Cell viability was routinely >95% and the cells appeared as a homogeneous population of rounded cells that assumed a flattened fibroblastic appearance and grew as a dispersed monolayer in culture. Granulosa cell and monocyte contamination following this isolation procedure is <1%.

To determine the effect of different monosaccharides on cell proliferation and androstenedione production, theca cells were plated at a density of 75 000 viable cells per well in 96-well flat-bottomed plates (Nunclon; Nunc, Roskilde, Denmark) in 250 μL glucose-free medium (see below). To determine the effect of the different monosaccharides on the mRNA expression of specific members of the solute carrier family, theca cells were plated at an equivalent density of 450 000 cells per well in 24-well flat-bottomed plates (Nunclon; Nunc) in 1 mL medium. The medium used to culture theca cells was Dulbecco's minimum essential medium : Ham's F12 (1 : 1; DMEM-F12), which was custom prepared to contain no glucose and a small amount of pyruvate (0.56 mM; JRH Biosciences, Lenexa, KA, USA; and Bio-Concept, Allschwil, Switzerland) and was subsequently supplemented with 0, 1.1, 2.1, 4.2, 8.4, 16.7 nM glucose, fructose or galactose. These doses were designed to span the normal physiological range of glucose in sheep blood and follicular fluid (1.1–2.1 mM; Somchit *et al.* 2007; Nandi *et al.* 2008;

**Table 1. Quantitative polymerase chain reaction primers used for semiquantification of *SCL2A* expression in ovine theca cells cultured in the presence of different monosaccharides**

| *SLC2A* | Primer | Reference |
|---|---|---|
| *SLC2A1* | Sense: 5′-TTAACCGCAACGAGGAGAAC-3′<br>Antisense: 5′-AACAGCTCCAGGATGGTGAC-3′ | Accession no. U89029 |
| *SLC2A4* | Sense: 5′-GGAGCTGGTGTGGTCACACA-3′<br>Antisense: 5′-GGAGCAGAGCCACAGTCATCA-3′ | Benomar *et al.* (2006) |
| *SLC2A5* | Sense: 5′-AGTCATCTCCATCATCGTCCT-3′<br>Antisense: 5′-GTACCCGCCACCATGTAGGCAG-3′ | Rizos *et al.* (2004) |
| *SLC2A8* | Sense: 5′-CTGGCATCTACAAGCCCTTC-3′<br>Antisense: 5′-TGAACTTGGCCTCCTCAAAG-3′ | Accession no. AF 495799 |

Scaramuzzi *et al.* 2010) up to a maximum of 16.7 mM, which represents the normal level of glucose supplementation in commercial preparations of this medium. The DMEM-F12 was also supplemented with sodium bicarbonate (2.2 mg mL$^{-1}$), HEPES (15 mM), bovine serum albumin (BSA; 1 mg mL$^{-1}$), Penstrep (100 U mL$^{-1}$ penicillin, 0.1 mg mL$^{-1}$ streptomycin), L-glutamine (3 mM), transferrin (2.5 μg mL$^{-1}$), selenium (0.1 μg mL$^{-1}$), insulin (10 ng mL$^{-1}$), longR3-insulin-like growth factor 1 (LR3-IGF1) (10 ng mL$^{-1}$) and 0.1 ng mL$^{-1}$ ovine (o) LH (NIDDK-oLH-26). This combination of insulin, IGF-1 and LH has been shown to be the optimum for the induction of maximal androstenedione production after approximately 6 days in culture (Campbell *et al.* 1998), and the addition of lipoproteins has no additional benefits on the level of steroid production (B. K. Campbell and R. Webb, unpubl. obs.), indicating that substrate supply is not a limiting factor.

For cultures in 96-well plates, each treatment was replicated in quadruplicate. The medium was changed every 48 h with 175 μL (70%) fresh medium, and cultures were maintained for 192 h. Spent medium was stored at −20°C until assay of androstenedione. Viable cell numbers at the end of the culture were estimated using neutral red staining, as described previously (Campbell *et al.* 1996). The sensitivity of this method was 2000 cells per well with an interassay CV of <5%. Steroid production per cell was calculated for the final time point. Each experiment was repeated at least three times. For cultures in 24-well plates for isolation of mRNA, physiological (2.1 mM) and supraphysiological (16.7 mM) doses of each monosaccharide were replicated in duplicate per treatment per time point, with the medium being exchanged every 48 h with equivalent fresh medium. Cell lysates for the analysis of *SLCA2* expression were collected for each time point and stored frozen in RNA lysis transfer (RLT) buffer with 1% β-mercaptoethanol before analysis. Spent medium was retained and stored at −20°C for subsequent analysis of androstenedione concentrations. Each experiment was repeated at least three times and results are expressed relative to a control sample taken from the pool of freshly isolated theca cells at the beginning of culture.

### *Assays of spent culture medium*

Concentrations of androstenedione in unextracted culture media were determined using a previously described radioimmunoassay (Campbell *et al.* 1998). The sensitivity of the assay was 18 pg androstenedione per tube and the intra- and interassay CV were <10%. Concentrations of lactate in the culture media were determined using a commercially available enzymatic assay kit for L-lactate (Randox, Belfast, Co. Antrim, UK) using a clinical chemistry autoanalyser (RX Imola; Randox), run with sample volumes increasing from 2 to 20 μL to increase sensitivity. The sensitivity of the assay was 0.043 mM. The intra- and interassay CV were <1%.

### *Expression of* SLC2A

First-strand cDNA libraries were synthesised using the IT 1st Strand Synthesis kit (ABgene, Epsom, UK) using total RNA extracted from ovine theca cells by the RNeasy mini kit (Qiagen, Valencia, CA, USA). These were semiquantified by real-time PCR using the 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA), with samples being amplified in triplicate for determination of expression of *SLC2A1*, *SLC2A4*, *SLC2A5* and *SLC2A8* using primers and PCR conditions described previously (Campbell *et al.* 2010*b*). Arbitrary *SLC2A* expression was normalised against starting amount differences using an *18s* endogenous control. The primers used in the present study are given in Table 1.

### *Data analysis*

Each experiment was repeated at least three times and the significance of treatment effects was determined by analysis of variance using either SPSS (SPSS UK, Surrey, UK) or Systat (SSI, San Jose, CA, US). The primary endpoints analysed were hormone production per 48 h at four time points (48, 96, 144 and 192 h), expressed as total mass of hormone after correction for total volume, the number of viable cells at the end of culture and the mass of hormone produced per 1000 cells over the final 48 h of culture. Data are presented as the least squares mean ± s.e.m. The normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test) of the data were determined using the appropriate tests and data were log transformed as required before analysis.

## Results

### *Effect of dose and type of monosaccharide on cell numbers, as well as androstenedione and lactate production*

#### *Induction profile*

Regardless of dose or type of monosaccharide, hormone production by theca cells from small follicles exposed to low

doses of LH (0.1 ng mL$^{-1}$) exhibited a typical induction profile for androstenedione (Campbell *et al.* 1998) characterised by a highly significant increase in the production of androstenedione ($P < 0.001$) with time in culture to a peak at 144 h culture (Fig. 1). However, the magnitude of peak production differed depending on the type and dose of monosaccharide, with fructose (Fig. 1*a*) resulting in markedly lower ($P < 0.001$) production than either galactose of glucose, which did not differ (Fig. 1*b*, *c*). Furthermore, for fructose, a supraphysiological dose (16.7 mM) resulted in higher levels of androstenedione production (Fig. 1*a*) than doses in the physiological range (2.1 mM), whereas the converse was true for glucose, with the physiological dose resulting in significantly higher ($P < 0.05$) peak production (Fig. 1*c*). Galactose produced a similar profile to glucose, but there was no significant difference in peak production of androstenedione between the physiological and supraphysiological doses.

### *Cell number*

Absence of monosaccharide in the culture media resulted in an approximate 50% suppression in the number of viable cells after 192 h culture ($P < 0.001$; Fig. 2) compared with cells cultured in the presence of monosaccharide. Excluding this effect of zero monosaccharide, there was no significant effect of dose (1.1–16.7 nM) within different types of monosaccharide and no significant sugar × dose interaction. Thus, cell number data were pooled within dose for each monosaccharide. The type of monosaccharide had a significant effect on cell number, with cultures in which fructose was supplied as an energy source having significantly ($P < 0.05$) fewer cells at the end of culture compared with those cultured in the presence of glucose or galactose, which did not differ (Fig. 2).

### *Androstenedione production*

Dose and type of monosaccharide had a highly significant effect on peak production of androstenedione after 144 h culture ($P < 0.001$), and there was a highly significant interaction between these two parameters ($P < 0.001$). Absence of a source of energy resulted in low levels of androstenedione production (Fig. 3). Over all doses, culture of theca cells in the presence of fructose resulted in significantly ($P < 0.001$) lower concentrations of androstenedione ($13.5 \pm 0.8$ ng mL$^{-1}$) than following culture in either galactose or glucose, which did not differ ($26.8 \pm 1.9$ and $26.3 \pm 1.4$ ng mL$^{-1}$, respectively). Examination of the dose–response data (Fig. 3) shows that increasing doses of fructose resulted in an increase in androstenedione production (Fig. 3*a*; $P < 0.05$), whereas the highest supraphysiological dose (16.7 mM) of glucose, but not galactose, was inhibitory (Fig. 3*b*, *c*; $P < 0.05$).

Analysis of androstenedione production expressed on the basis of cell number at the end of culture revealed highly significant effects of dose and type of monosaccharide, and a highly significant interaction between these two parameters ($P < 0.001$). Over all doses, the culture of theca cells in the presence of fructose resulted in significantly ($P < 0.01$) lower levels of androstenedione production ($5.2 \pm 0.1$ log ng per $10^3$ cells) than culture in either galactose or glucose, which did not differ significantly ($5.6 \pm 0.1$ and $5.9 \pm 0.1$ log ng per $10^3$ cells,

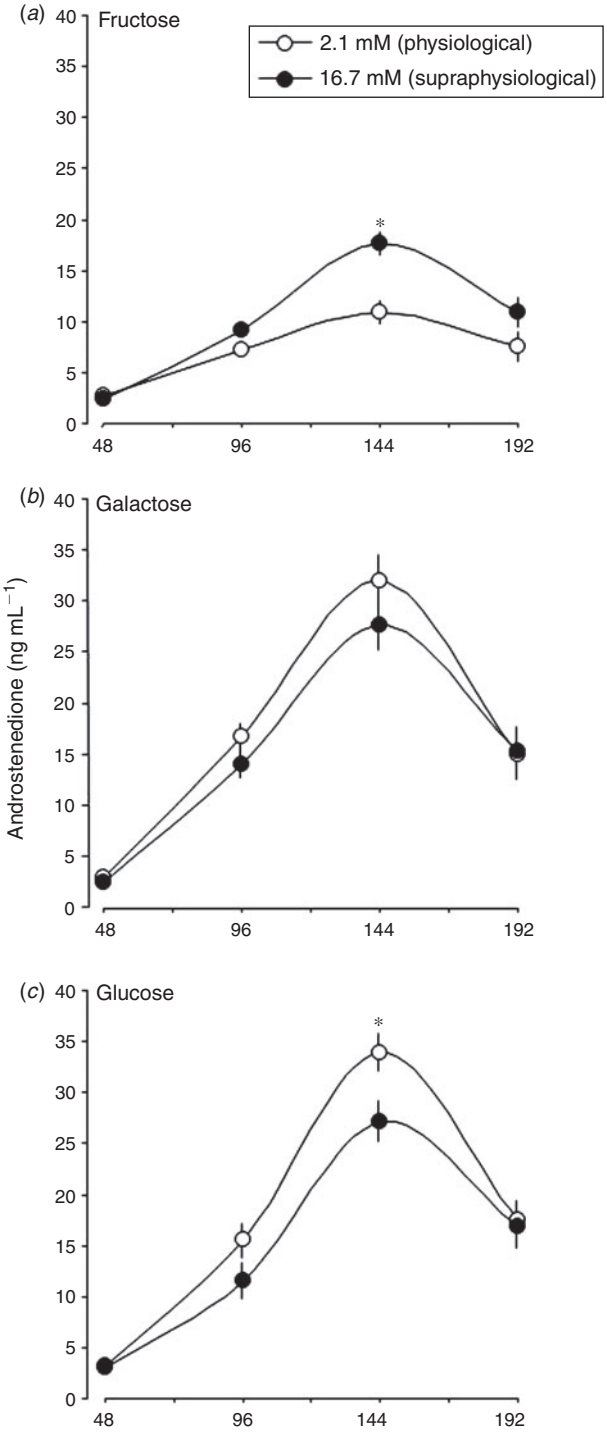


**Fig. 1.** Induction profiles of androstenedione production by ovine theca cells isolated from small antral follicles cultured under conditions know to induce cellular differentiation (0.1 ng mL$^{-1}$ LH, 10 ng mL$^{-1}$ insulin and 10 ng mL$^{-1}$ LR3-IGF1) with increasing time in culture. Cells were cultured in the presence of either a physiological (2.1 mM) or supraphysiological (16.7 mM) dose of each of the three monosaccharides: (*a*) fructose, (*b*) galactose and (*c*) glucose. The supraphysiological dose equates to the dose of glucose commonly used in culture media. Values are the mean ± s.e.m. *$P < 0.05$ for comparisons between doses at each time point for each sugar.

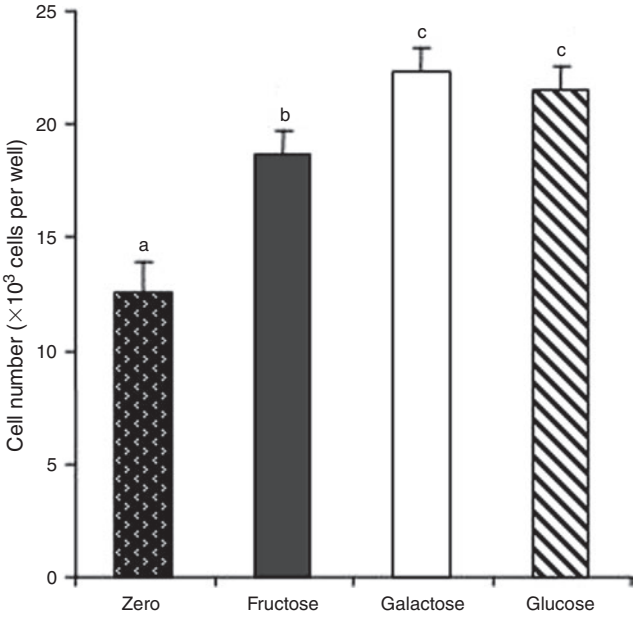


**Fig. 2.** Effects of culturing ovine theca cells from small antral follicles under conditions known to induce cellular differentiation (0.1 ng mL$^{-1}$ LH, 10 ng mL$^{-1}$ insulin and 10 ng mL$^{-1}$ LR3-IGF1) in the absence (0) or presence of the three monosaccharides (fructose, galactose and glucose) on cell number at the end of culture (192 h). Data across all doses for each monosaccharide have been pooled. Values are the mean ± s.e.m. Columns with different letters differ significantly ($P < 0.05$).

respectively). The dose–response data for androstenedione, when corrected for cell number, showed a positive relationship with fructose (Fig. 4*a*), but not with galactose (Fig. 4*b*). Similarly, although a significant depression in cell-corrected androstenedione production was observed between the 1.1 and 8.4 mM doses of glucose (Fig. 4*c*), at the higher dose of 16.7 mM the level of production increased and was not different from that at the lower doses (1.1 and 2.1 mM).

### *Lactate production*

Lactate production by theca cells was very low compared with that of granulosa cells cultured under similar conditions (Campbell *et al.* 2010*b*). Overall, there were no effects of time in culture and dose or type of monosaccharide on the production of lactate, with the overall mean concentrations of lactacte produced being 43.1 ± 0.9, 42.7 ± 0.9 and 42.6 μg dL$^{-1}$ for glucose, galactose and fructose, respectively, across all time points.

### *Expression of* SLC2A *mRNAs*

For *SLC2A5* mRNA, a standard curve was successfully constructed using cDNAs from samples of sheep intestine, but no positive signal was detected in freshly isolated or cultured theca cells. Conversely, mRNA expression for *SLC2A1*, *SLC2A4* and *SLC2A8* was detected in fresh and cultured theca cells, and the type and dose of monosaccharide had highly significant ($P < 0.01$) effects on the expression of each transporter. At the physiological dose of 2.1 mM, the type of monosaccharide had

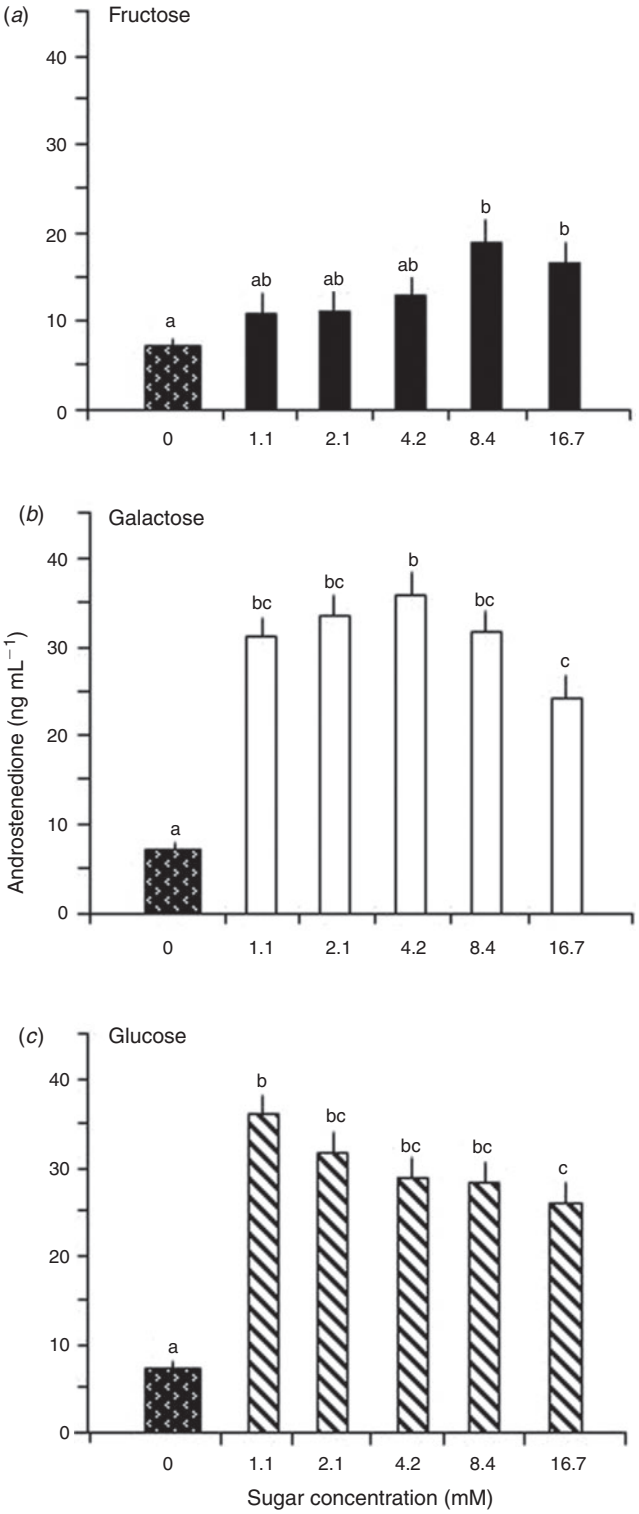


**Fig. 3.** Effects of culturing ovine theca cells from small immature follicles in the presence of increasing doses of the three monosaccharides on androstenedione production at peak levels after 144 h culture. (*a*) Fructose, (*b*) galactose and (*c*) glucose. Values are the mean ± s.e.m. Columns with different letters differ significantly ($P < 0.05$).

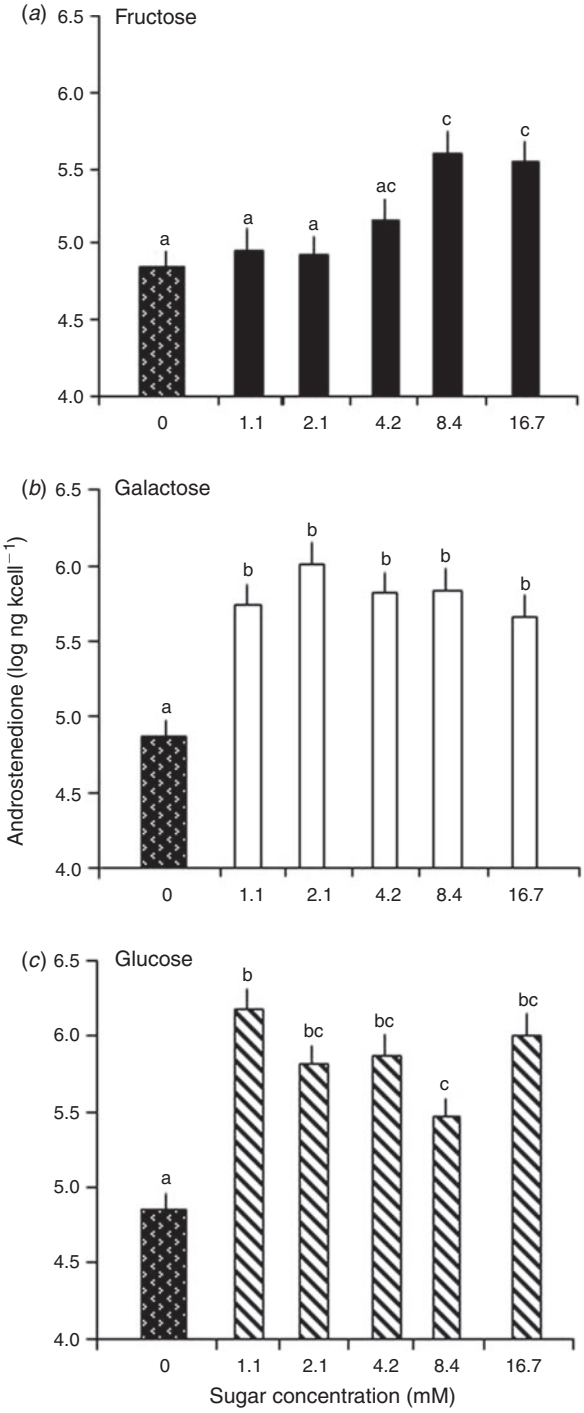


**Fig. 4.** Androstenedione production by thecal cells at the end of culture (192 h) expressed on a per cell basis following culture in the presence of increasing doses of (*a*) fructose, (*b*) galactose and (*c*) glucose. Values are the mean ± s.e.m. Columns with different letters differ significantly ($P < 0.05$).

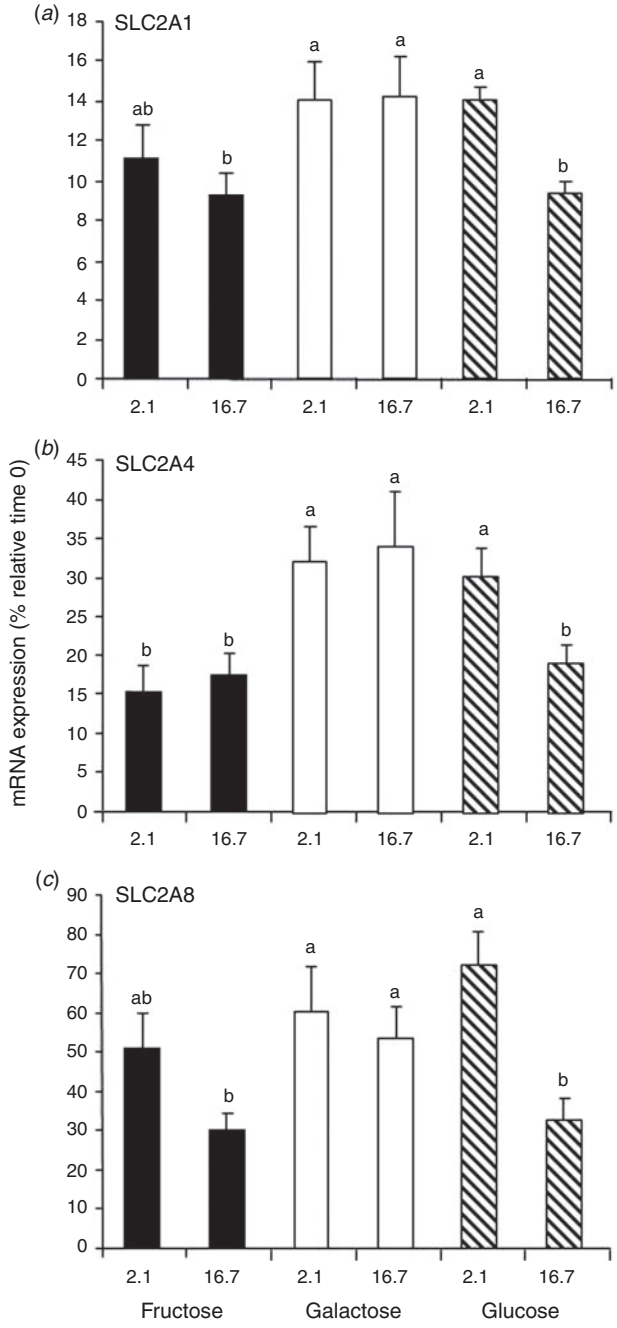


**Fig. 5.** Effects of culturing theca cells for 144 h in the presence of physiological (2.1 mM) or supraphysiological (16.7 mM) doses of fructose, galactose and glucose on the mRNA expression of the glucose transporters (*a*) SLC2A1, (*b*) SLC2A4 and (*c*) SLC2A8. Data are expressed as a percentage of the level of expression of the relevant SLC2A in freshly isolated theca cells before culture (Time 0). Values are the mean ± s.e.m. Columns with different letters differ significantly ($P < 0.05$).

no significant effect on the level of expression of any of the SLC2As, with the exception of *SLC2A4* expression in the presence of low-dose fructose (Fig. 5). These cells had significantly ($P < 0.05$) lower levels of expression than cells cultured in the same dose of galactose or glucose. Relative to the physiological dose, the supraphysiological dose of 16.7 mM glucose, commonly used in many commercial culture media, significantly reduced ($P < 0.05$) the level of thecal cell expression of

all three SLC2As measured (*SLC2A1*, *SLC2A4* and *SLC2A8*). In contrast, the supraphysiological dose of both fructose and galactose had no significant effect on *SLC2A* expression (Fig. 5).

## Discussion

The results of the present study show that a readily metabolised energy source is required to support the gonadotrophin-induced differentiation of theca cells and that the three monosaccharides tested varied markedly in their ability to support this process. Thus, of the monosaccharides evaluated, glucose and galactose were equally effective, whereas fructose was markedly inferior. It is likely that this difference can be attributed to the fact that expression of the fructose-specific *SCL2A5* could not be detected in ovine theca cells, whereas expression of glucose and galactose transporters (*SLC2A1*, *SLC2A4* and *SLC2A8*) was observed. However, differences between glucose and galactose effects were evident in that supraphysiological doses of glucose, similar to those used routinely in cell culture media, were found to be inhibitory to thecal cell differentiation and SLC2A expression, suggesting that glucose functions at a local level to regulate thecal cell function. Importantly, the results of the present study differ markedly from those reported previously in a parallel series of experiments using ovine granulosa cells (Campbell *et al.* 2010*b*), and it is clear that fundamental differences exist between these two follicular cell types in terms of the potential substrates and metabolic pathways used to support gonadotrophin-induced differentiation.

In ovine granulosa cells, we observed previously that glucose, metabolised under anoxic conditions to lactate, is the preferred energy substrate to support FSH-induced differentiation of granulosa cells and that fructose and pyruvate, but not galactose, are alternative energy substrates (Campbell *et al.* 2010*b*). The results of the present study indicate several fundamental differences between granulosa and theca cells in relation to their ability to metabolise monosaccharides: (1) theca cells can readily metabolise galactose, whereas granulosa cells cannot; (2) granulosa cells can readily metabolise fructose, whereas theca cells cannot; and (3) although both cell types use glucose, cultured granulosa cells metabolised this substrate anaerobically and accumulated large amounts of lactate in the culture medium, whereas cultured theca cells did not. Clearly, within a follicle the membrana granulosa cell layer is avascular with low oxygen tensions (Murray *et al.* 2009), whereas the theca is highly vascularised (Magoffin 2005) and it is probable that these differences in oxidative metabolism between the cell types reflects fundamental differences in metabolic pathways that are related to differences in oxygen tension in the granulosa and thecal cell layers of the follicle. However, it is interesting to note that although pyruvate is as effective as glucose in supporting the FSH-induced differentiation of cultured ovine granulosa cells, these cells do not accumulate lactate if pyruvate is provided as a sole energy source (Campbell *et al.* 2010*b*). We have suggested previously that this anomaly may be explained by the differential activity of the monocarboxylate transporters (MCT) that transfer pyruvate and lactate across cell membranes (Merezhinskaya and Fishbein 2009), and we now also suggest that differences in the expression of MCT could explain the differences in oxidative metabolism that we have observed between granulosa and theca cells. Further work is required to test these hypotheses.

Once absorbed, fructose can enter the glycolytic pathway directly by conversion to fructose-6-phosphate by hexokinases, or it can be converted into fructose-1-phosphate by fructokinase from which the trioses, dihydroxyacetone (DHAP) and glyceraldehyde are synthesised before complete metabolism to pyruvate (Zubay 1998). The relative inability of theca cells to use fructose as an energy source is consistent with the fact that we have been unable to detect the mRNA for the fructose transporter SCL2A5 in theca cells. This interpretation is supported by the observation that there was a positive relationship between the dose of fructose and androstenedione production, so it is possible that only at high levels of exposure was there sufficient fructose entering the cells to provide the energy required to support cellular differentiation. However, we have shown previously that granulosa cells can use fructose to support FSH-induced aromatase activity, despite the fact that we were unable to detect *SCL2A5* mRNA expression in granulosa cells (Campbell *et al.* 2010*b*). It would therefore appear likely that granulosa cells may express other SLC2As capable of transporting fructose, such as SLC2A2, SLC2A7 and SLC2A11 (Zhao and Keating 2007), which may not be expressed by theca cells. Again, further work is required to evaluate this hypothesis. Overall, the results of the present study indicate that fructose is unlikely to have a direct effect on thecal cell function and this interpretation is consistent with our recent *in vivo* studies involving infusion of fructose, where we concluded that fructose modulated ovarian function through indirect effects on insulin and/or gonadotrophin release (Campbell *et al.* 2010*a*).

The main products of galactose metabolism are glucose (in the form of glucose-1-phosphate, which then enters the glycolytic pathway) and uridine diphosphate (UDP)-galactose, which is required for glycol conjugation of proteins and lipids (Forges *et al.* 2006). Several SLC2As that transport galactose (SLC2A1, SLC2A2, SLC2A3 and SLC2A8) have also been reported in the ruminant ovary (SLC2A1 and SLC2A3: Nishimoto *et al.* 2006; SLC2A1, SLC2A3 and SLC2A8: Pisani *et al.* 2008; SLC2A1: Williams *et al.* 2001; and SLC2A1 and SLC2A8: Campbell *et al.* 2010*b*). It is perhaps therefore not surprising that in the present study galactose was used as readily as glucose to support LH-induced thecal cell differentiation. However, several lines of evidence suggest that galactose is inhibitory to ovarian function. Thus, in addition to not being metabolised by ovine granulosa cells (Campbell *et al.* 2010*b*), we have shown that direct ovarian infusion of galactose into sheep with ovarian autotransplants results in the suppression of both androstenedione and oestradiol secretion (Campbell *et al.* 2010*b*). Furthermore, in humans, the accumulation of galactose or its metabolites is associated with impaired ovarian function that, in addition to being due to direct ovarian toxicity, can be attributed to the deficient galactosylation of glycoproteins and glycolipids, the induction of apoptosis and the modulation of the expression of intra-ovarian growth factors such as growth differentiation factor-9 (Forges *et al.* 2006). The absence of inhibitory effects of galactose on thecal cell function in the present study, even at supraphysiological doses, suggests that the detrimental effects

of this monosaccharide on ovarian function are mediated by direct effects on the function of the avascular regions of antral follicles, such as the membrana granulosa and the cumulus–oocyte complex, rather than the vascularised theca.

One surprising aspect of the results of the present study was the inhibitory effect of supraphysiological doses of glucose on thecal cell differentiation. As observed previously in granulosa cells (Campbell *et al.* 2010*a*), doses of glucose in the physiological range (1.1–2.1 mM; Somchit *et al.* 2007; Nandi *et al.* 2008; Scaramuzzi *et al.* 2010) were highly effective in supporting gonadotrophin-induced differentiation but, unlike granulosa cells, the 10-fold higher dose (16.7 mM) commonly found in many commercial culture media was actually inhibitory in theca cells. In addition to suggesting that these supraphysiological doses of glucose in commercial media are unnecessary and potentially deleterious, this finding supports the existence of functional glucose-sensing mechanisms in these cultured cells. An example of such a mechanism is the hexosamine signalling pathway, which, through the flux of glucose and the synthesis of glucosamine-6-phosphate, is able to sense and respond to nutritional fluctuations by altering specific gene expression (Marshall *et al.* 1991; Obici and Rossetti 2003; Curi *et al.* 2005). The presence of glucose-sensing mechanisms is also consistent with the observed reduction in the expression of *SLC2A1*, *SLC2A4* and *SLC2A8* by theca cells in response to high doses of glucose seen in the present series of experiments. An inhibitory effect of glucose on SLC2A expression has been widely observed (for a review, see Klip *et al.* 1994). although in cultured cells reduced expression of *SLC2A1*, rather than *SLC2A4*, has been most commonly noted. However, in cultured ovine granulosa cells we have also observed that high doses of glucose and fructose suppress both *SLC2A1* and *SLC2A4*, although no effect on *SLC2A8* was observed (Campbell *et al.* 2010*b*). Thus, apart from these effects on the expression of *SLC2A8*, there was good agreement in terms of the inhibitory effects of high doses of fructose and glucose on the expression of *SLC2A1* and *SLC2A4* in both granulosa and theca cells. However, in the present series of experiments, it is also possible that the fall in mRNA expression in theca cells cultured in the presence of fructose was also due, at least in part, to a fall in cell viability noted in these cultures. Overall, these results suggest that the homeostatic local regulatory mechanisms that suppress glucose transporters in ovarian cell types in response to high doses of sugars are specific for cell type, monosaccharide type and type of SLC2A, and thus may represent a complex but important local mechanism regulating the metabolism of ovarian somatic cells.

In conclusion, the results of the present study show that glucose and galactose, but not fructose, are readily metabolised via oxidative pathways to support the LH-induced differentiation of cultured theca cells. These differences in monosaccharide utilisation appear to be related to relative patterns of expression of mRNAs specific for members of the SCL2A family in that there was no expression of the fructose-specific transporter *SCL2A5*, whereas expression for the glucose and galactose transporters (*SCL2A1*, *SCL2A4*, *SCL2A8*) was present. Finally, these results show that large doses of glucose, similar to those routinely used in commercial media for cell culture, are inhibitory to thecal cell differentiation and to the expression of *SCL2A1*, *SCL2A4* and *SCL2A8*, thus providing a mechanism whereby glucose, but not galactose, can directly modulate ovarian function. Therefore, the present study has provided clear evidence that the energy sources and metabolic pathways used by cultured ovine theca cells to support gonadotrophin-induced cellular differentiation differ markedly from those reported previously for granulosa cells (Campbell *et al.* 2010*b*). These findings suggest that the established effects of nutrition on reproductive function are mediated, at least in part, by direct effects at the level of the ovary, although further work is required to determine the underlying mechanisms.

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