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Androgenic Control of 1-Alkyl-2,3-Diacylglycerol in the Harderian Gland of the Golden Hamster, *Mesocricetus auratus*[1]

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Harderian glands of golden hamsters produce a copious lipid secretion, most of which is in the form of 1-alkyl-2,3-diacylglycerol (ADG). Sexual differences are seen in the composition of golden hamster ADG and in the morphology of secretory lipid droplet. ADGs from females contained abundant *iso*- and *anteiso*-branched chain alkyl groups and fatty acids [Seyama, Y., Otsuka, H., Ohashi, K., Vivien-Roels, B., and Pevet, P. (1995) *J. Biochem.* 117, 661-670]. Female hamsters were either untreated or given subcutaneous testosterone pellets. Treatment of females with testosterone led to the disappearance of such branched chain alkyl groups and fatty acids. Intact males had ADGs with entirely saturated straight chain alkyl groups and fatty acids. Castration led to the appearance of *iso*- and *anteiso*-branched chain alkyl groups and fatty acids. These observations suggested that the production of branched chain fatty acids in the Harderian gland of golden hamster is inhibited by testosterone at the step of isovaleryl-CoA dehydrogenase and 2-methyl branched-chain acyl-CoA dehydrogenase.

Key words: acyl-CoA dehydrogenase, alkyldiacylglycerol, androgen, golden hamster, Harderian gland.

Harderian glands are accessory lacrimal glands, present in the medial portion of the orbit in most terrestrial vertebrates. They are particularly prominent in rodents, where they may be the largest structure in the orbit. In mammals they are unusual amongst exocrine glands in that they produce a lipid secretion which is released by exocytosis (*1, 2*). The function of this lipid secretion is the subject of speculation; some of the current theories are that they lubricate the third eyelid, are solvents for pheromones, are involved in thermoregulation, are solvents of bioactive substances, or have bacteriocidal effects (*3*).

The lipid product of the rodent Harderian gland is also unusual in that most of this lipid is in the form of 1-alkyl-2,3-diacylglycerol (ADG) in rabbits (*4, 5*), guinea pigs (*6, 7*), and golden hamsters (*8, 9*). Harderian glands of golden (Syrian) hamster exhibit many differences associated with gender, and most of these are controlled by the endocrine state of the animal. The type of secretory lipid droplets is amongst these dimorphic features: female hamsters contain an overwhelming preponderance of cells with small lipid droplets (type I cells), whereas males contain both type I cells and cells with large lipid droplets (type II cells). Castration of male hamsters leads to the disappearance of type I cells, whereas treatment of females with testosterone leads to the appearance of type II cells (*10-13*).

Lipids in the hamster Harderian gland were studied by Lin and Nadakavukaren (*14*) and, more recently, by Murawski *et al.* (*8*) and Seyama *et al.* (*9*). Most of this lipid is ADG. Males show three broad classes of ADG on thin layer chromatography, whereas females show two classes (*8, 9*). Lin and Nadakavukaren (*14*) claimed that males have shorter chain fatty acids than females, most of whose fatty acids are unsaturated. However, both Murawski *et al.* (*8*) and Seyama *et al.* (*9*) showed that the techniques used in the earlier study were inadequate for the material and that unsaturated fatty acids are not present in hamster Harderian lipid. Male ADGs have predominantly straight chain fatty acids and alkyl groups, whereas female ADGs also contain *iso*- and *anteiso*-branched acyl and alkyl groups (*8, 9*).

Thus, male hamster Harderian glands have both large and small lipid droplets and entirely straight-chain alkyl and acyl groups, whereas females have only small lipid droplets and have both straight- and branched-chain alkyl and acyl groups (*9, 13*). The size and appearance of lipid droplets are controlled by androgens (*13*). This study was designed to determine the effects of androgen treatments on the composition of ADGs in male and female hamster Harderian glands.

MATERIALS AND METHODS

Animals—Golden hamsters (*Mesocricetus auratus*) were obtained from Charles River, Canada, and were housed in a vivarium and allowed food and water *ad libitum*. Housing

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Abbreviations: ADG, 1-alkyl-2,3-diacylglycerol; TLC, thin layer chromatography; GLC, gas-liquid chromatography.

conditions included controlled temperatures (20±2°C) and a photoperiod of 14 h light/day (lights on at 06:00 h).

Female Treatments—Females were untreated ($n=6$) or given pellets containing testosterone ($n=6$). Beeswax pellets containing testosterone (*13*) were inserted subcutaneously at the nape of the neck at the beginning of the experiment and replaced with freshly prepared pellets fortnightly. Female hamsters were examined during four consecutive days for the presence of vaginal mucus, indicative of estrus (*15*). Sampling was done between 9 and 9½ weeks after the beginning of the experiment; all were sampled in the morning. At sampling, animals were decapitated and serum was prepared for hormonal analysis described in Ref. *13*. The Harderian glands were dissected from the orbits and quickly frozen on dry ice. A portion of each gland was preserved for morphometric studies, which have been reported elsewhere (*13*).

Male Treatments—Males were intact ($n=6$) or castrated *via* the scrotal route ($n=6$). Animals were kept for 8½ weeks before being sampled. Sampling was done in the same way as for the females.

Extraction of Lipids—Crude lipid extracts were made as follows: frozen glands were weighed and homogenized in 4 ml of chloroform/methanol (2 : 1, v/v) by use of a Polytron. They were left overnight at room temperature and then vortexed and filtered into preweighed tubes. Residual lipids were washed out with chloroform/methanol, which was also filtered. The liquid phase was then evaporated in a stream of nitrogen and the tubes containing lipids were weighed. There were no significant differences in the lipid contents among these four groups.

Preparation of Alkyldiacylglycerols—Silica SEP-PAK cartridge (Waters, No. 51900) was prewashed with 5 ml of hexane. Crude lipids (about 4 mg) were dissolved in a small amount of hexane and applied to the SEP-PAK cartridge, which was then washed with 20 ml of hexane/benzene (8 : 2, v/v). Then 30 ml of hexane/benzene (4 : 6, v/v) was used to elute the ADGs; in addition, cholesterol was obtained in a 10-ml benzene fraction, and polar lipids (*e.g.* phospholipids) were eluted in a 15-ml methanol fraction.

Thin Layer Chromatography (TLC)—Silica gel TLC plates (Silica Gel 60, Merck) were used with hexane/diethylether/acetic acid (80 : 20 : 1, v/v/v) as a developing solvent. The spots were located by heating at 120°C after spraying with 20% H_2SO_4.

Preparation of Fatty Acid Methyl Esters and Isopropylidene Derivatives—ADG was heated at 100°C for 2 h with 0.5 ml of 3% anhydrous HCl in methanol in a sealed tube. Fatty acid methyl esters were extracted with three 1-ml portions of hexane. The residue in the tube was evaporated to dryness and treated with 1 ml of acetone and 2 μl of 70% perchloric acid. After 30 min, the solution was made basic by the addition of excess ammonium hydroxide and evaporated to dryness. The residue was then dissolved in chloroform and the solution was washed with water and evaporated to dryness, leaving the isopropylidene derivatives (*i.e.*, the alkyl groups) of ADGs.

Gas-Liquid Chromatography (GLC)—GLC was performed in a Shimadzu GC-14A gas chromatograph fitted with a flame ionization detector and a glass capillary column (0.28 mm×15 m) coated with CARBOWAX 20M (Hewlett Packard). The carrier gas was N_2 at a flow rate of 1 ml/min. The scavenge gas flow was 50 ml/min. In the case of fatty acid methyl esters, the column temperature was kept at 200°C (isothermal) for 35 min. In the analysis of isopropylidene derivatives of alkylglycerols, the column temperature was kept at 180°C for 2 min and then increased to 220°C at a rate of 5°C/min. Compositions of individual fatty acids and isopropylidene derivatives were calculated as percentages of the total for each animal. They are expressed here as the mean±SEM of each group.

RESULTS

TLC of Harderian Gland ADGs—Thin layer chromatograms (Fig. 1) from lipid extracts of intact male hamsters (lane 1) showed ADGs as an elongated spot which could be resolved into three overlapping spots. Castrated male hamsters (lane 2) showed two distinct spots, similar to those seen intact females.

Intact female hamsters (lane 3) showed two distinct spots, a faint upper spot and a lower intense spot. ADGs of female hamsters supplied with exogenous testosterone (lane 4) showed TLC patterns indistinguishable from those of intact males, with an elongated triple spot.

Alkyl Groups (Males)—Isopropylidene derivatives represent the alkyl groups at the C-1 position of ADG. Compositions of isopropylidene derivatives of alkyl groups in male ADGs are shown in Fig. 2. All of the alkyl groups in intact male hamsters consisted of saturated straight chains (Fig. 2a). All of the saturated straight chain alkyl groups between C14:0 and C22:0 were present, with the highest concentration being C18:0, and appreciable concentrations of C16:0, C17:0, C19:0, and C20:0. At 8½ weeks after castration, 47% of the alkyl groups were branched (Fig. 2b). Of the straight chains, C18:0 was still predominant. There was a significant increase in C20:0 and decreases in C15:0, C16:0, C17:0, C18:0, and C19:0 from those in intact

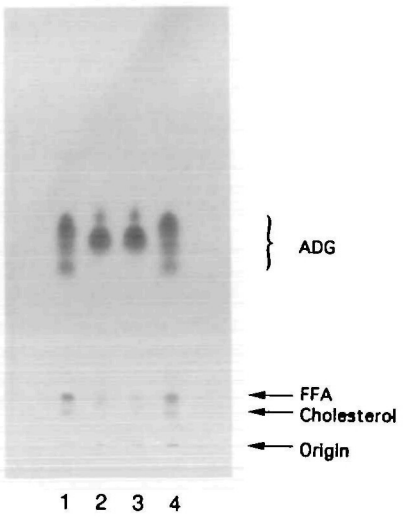

Fig. 1. **Thin-layer chromatogram of lipid in Harderian glands of golden hamsters.** A silica gel 60 plate (Merck, Darmstadt) was developed with hexane-diethylether-acetic acid (80 : 20 1, v/v/v). The spots were located by heating the plate at 120°C after spraying it with 20% sulfuric acid Samples: lane 1, crude lipid from glands of intact males; lane 2, castrated males; lane 3, intact females, lane 4, testosterone-treated females. ADG: 1-alkyl-2,3-diacylglycerol, FFA: free fatty acid.

controls (Table I). In fact, C15:0 was totally lacking in castrates. Amongst the branched chain alkyl groups, even-numbered *iso*-branched (C18 and C20) and odd-numbered *iso*- and *anteiso*-branched (C17, C19, C21) chains were present.

Alkyl Groups (Females)—In intact females, more than half of the alkyl groups were branched, and these were predominantly odd-numbered *iso*- and *anteiso*-branched chains (C17, C19, C21); they also including even-numbered *iso*-branched chains (C18 and C20) (Fig. 2c). Of the straight chain alkyl groups, most were C18:0 and C20:0, with very small amounts of other straight chain derivatives (Table I).

Testosterone treatment almost completely prevented the formation of any branched chain alkyl groups (Fig. 2d). Most of the straight chains were C18:0 and C19:0, with appreciable concentrations of C16:0, C17:0, C20:0, and C21:0 as well (Table I).

Fatty Acids (Males)—In intact male hamsters, Harderian fatty acids were almost entirely straight chain saturated ones (Fig. 3a). Most abundant were C14:0 and C15:0, with smaller, but still appreciable amounts of C16:0, C17:0, and C18:0 (Table II). Castration led to significant reductions in the percentages of all these straight chain fatty acids except C16:0 (which increased from about 14% to over 55% of all fatty acids) (Fig. 3b). Castration also led to the appearance of appreciable concentrations of branched chain fatty acids (to nearly 28% of all fatty acids). These included even chain-length *iso*-branched (C16 and C18) and odd chain-length *iso*- and *anteiso*-branched (C15, C17, C19) fatty acids (Table II).

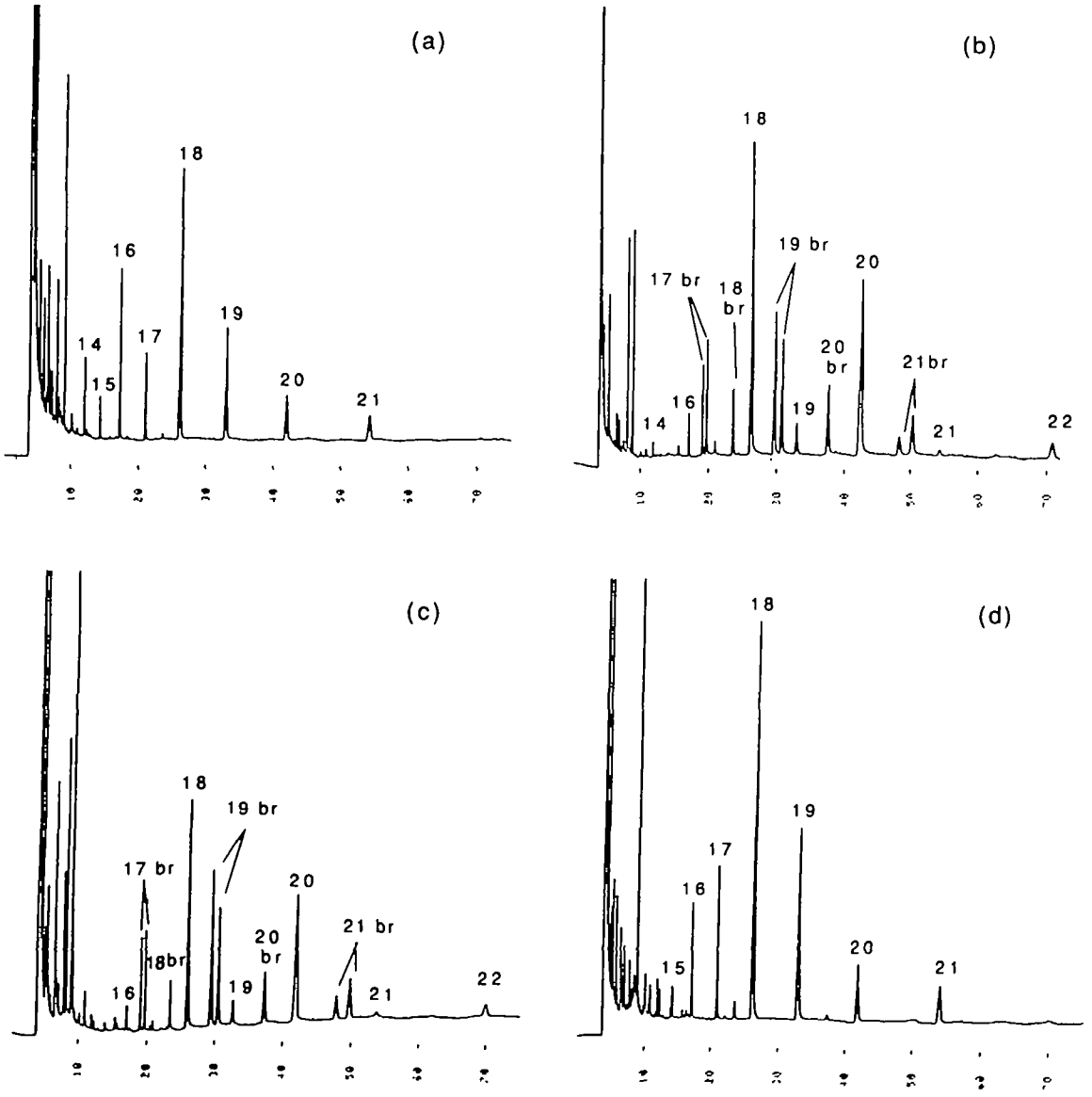

Fig. 2. **Gas chromatogram of isopropylidene derivatives of alkylglycerol derived from ADG from Harderian glands of golden hamsters.** (a) intact males; (b) castrated males; (c) intact females; (d) testosterone-treated females. Numbers (14-22) indicate the chain length of each peak; "14" means C14:0. The abbreviation, "br," means branched chain acid. Column: 0.28 mm × 15 m fused silica column coated with CARBOWAX 20M. The carrier gas was N_2, at the flow rate of 1 ml/min. The column temperature was kept at 180°C for 2 min and then increased to 220°C at the rate of 5°C/min.

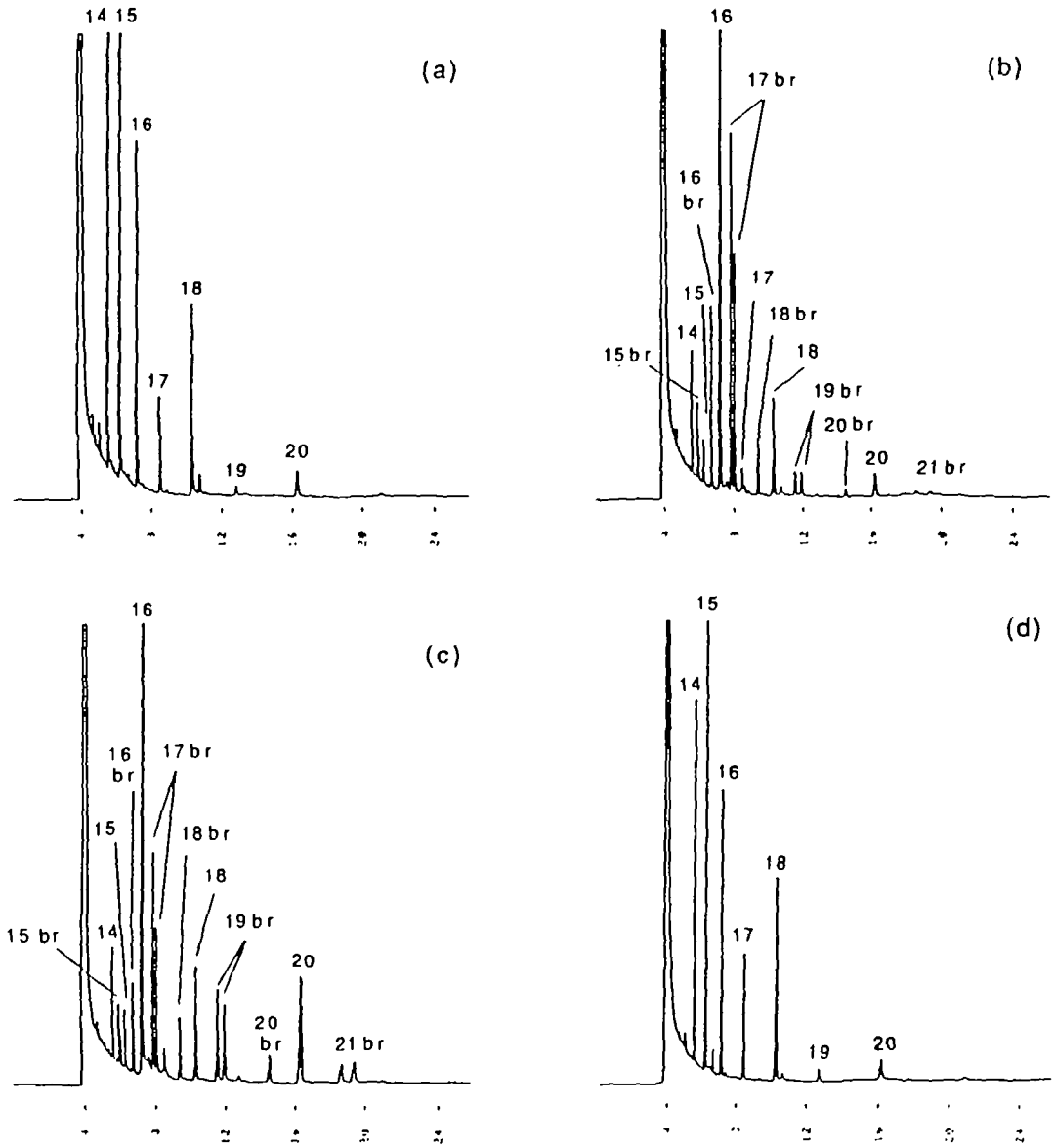

Fig. 3. **Gas chromatogram of fatty acid methyl esters of ADG.** (a) intact males; (b) castrated males; (c) intact females; (d) testosterone-treated females. Numbers (14-20) indicate the chain length of each peak; "14" means C14:0. The abbreviation, "br," means branched chain acid. The analytical conditions were the same as in Fig. 2 except for the column temperature (kept at 200°C).

Fatty Acids (Females)—Harderian glands of intact female hamsters were characterized by the presence of straight and branched chain fatty acids in a ratio of about 73:27 (Fig. 3c). Palmitic acid (C16:0) represented most of the fatty acids (about 55%), with smaller amounts of C18:0 and C20:0 and traces of other straight chain fatty acids (Table II). Amongst the branched chain fatty acids were even chain-length *iso*-branched (C16 and C18) and odd chain-length *iso*- and *anteiso*-branched (C15, C17, C19) fatty acids. Testosterone treatment of females led to the nearly total elimination of all branched chain fatty acids (Fig. 3d). There were also reductions in the percentage of C16:0 and C20:0 and significant increases in C17:0, C18:0 and, especially, in C15:0 and C14:0 (Table II).

DISCUSSION

The major secretory lipid present in the golden hamster Harderian gland is 1-alkyl-2,3-diacylglycerol (ADG). Like so many features of this gland in this hamster, the alkyl and acyl groups of ADG show sexual differences (*8, 9*). This study established that these differences in lipids have an endocrine basis and are regulated by testosterone at the step of catabolism of branched chain amino acids, leucine, isoleucine, and valine.

With regard to the characteristics of Harderian ADG in untreated hamsters, our findings were generally in agreement with those of previous workers (*8, 9*). Thus, male hamsters showed three overlapping spots of ADG on TLC; their alkyl and acyl groups were entirely straight saturated

TABLE I. **GLC determination of alkyl compositions of ADG in Harderian glands of golden hamsters.**

Alkyl (%)	Intact male ($n=6$)	Castrated male ($n=6$)	Intact female ($n=6$)	Testosterone-treated female ($n=6$)
14:0	6.9±0.8	1.3±0.4	0.1±0.1	1.2±0.6
15:0	3.3±0.4		2.4±1.5	1.2±0.4
16:0	17.5±1.2	2.9±0.8	1.1±0.2	5.4±1.2
17:0	7.9±0.6	1.1±0.3	1.8±1.1	9.2±0.8
18:0	37.8±1.8	27.0±3.8	23.9±1.9	41.0±5.0
19:0	14.0±1.9	2.7±0.3	1.3±0.4	25.6±3.8
20:0	8.5±0.8	17.3±1.3	17.0±1.0	7.4±1.2
21:0	4.0±0.7	0.1±0.1		8.1±1.9
22:0	0.1±0.1	1.0±0.5	0.7±0.4	
Odd numbered	29.2	3.9	5.5	44.0
Even numbered	70.8	49.5	42.7	55.1
Straight chain	100.0	53.4	48.3	99.1
16 is		0.4±0.2		
17 is		5.3±1.3	7.7±1.2	
17 ai		6.5±1.5	7.2±1.2	
18 is		4.3±0.5	2.8±0.6	0.5±0.2
19 is		11.3±0.8	15.4±0.5	
19 ai		9.2±0.7	9.9±0.4	
20 is		4.8±0.5	4.0±0.9	0.3±0.1
21 is		1.9±0.1	1.7±0.6	
21 ai		3.0±0.7	3.0±1.0	
is (even)	0.0	9.5	6.8	0.9
is (odd)	0.0	18.5	24.8	0.0
ai (odd)	0.0	18.6	20.1	0.0
Branched chain	0.0	46.6	51.7	0.9

is: *iso*-branched, ai: *anteiso*-branched. Values are mean±SEM.

TABLE II. **GLC determination of fatty compositions of ADG in Harderian glands of golden hamsters.**

Fatty acids (%)	Intact male ($n=6$)	Castrated male ($n=6$)	Intact female ($n=6$)	Testosterone-treated female ($n=6$)
12:0	3.1±0.9	0.8±0.4	0.3±0.1	0.9±0.3
13:0	1.6±0.3			1.5±0.2
14:0	30.2±1.3	5.2±1.5	2.2±0.3	17.2±2.0
15:0	30.4±1.7	2.2±0.3	1.4±0.1	44.1±4.0
16:0	14.3±0.9	55.1±3.1	55.4±2.1	12.2±0.5
17:0	4.4±0.3	1.6±0.2	1.2±0.2	6.8±1.0
18:0	11.1±1.3	4.7±0.3	5.6±0.4	13.9±1.9
18:1	1.4±0.6	0.3±0.2		0.2±0.1
19:0	0.7±0.1		0.2±0.1	0.7±0.3
20:0	2.6±0.4	2.4±0.9	6.8±0.8	1.6±0.3
Odd numbered	37.2	3.8	2.8	53.0
Even numbered	61.4	68.2	70.2	45.8
Straight chain	99.9	72.3	73.0	99.1
15 is		2.2±0.4	1.1±0.1	
15 ai		0.7±0.0	0.4±0.1	
16 is	0.1±0.1	3.9±0.4	1.8±0.2	0.9±0.1
17 is		10.1±0.8	8.3±0.8	
17 ai		6.6±0.5	4.5±0.2	
18 is		1.5±0.2	2.7±0.8	
19 is		1.3±0.5	4.8±0.6	
19 ai		1.2±0.4	3.2±0.4	
20 is		0.2±0.2	0.3±0.3	
is (even)	0.1	5.6	4.8	0.9
is (odd)	0.0	13.6	14.1	0.0
ai (odd)	0.0	8.4	8.1	0.0
Branched chain	0.1	27.7	27.0	0.9

is: *iso*-branched, ai: *anteiso*-branched. Values are mean±SEM.

chains, predominantly C18:0 and C16:0 (alkyl) and C15:0 and C14:0 (fatty acids) (*9*). Female glands showed two spots on TLC; both alkyl and acyl groups consisted of an appreciable proportion of branched chains, with the branching in both the *iso*- and *anteiso*-positions (*9*). *Iso*-branched alkyl and acyl groups had both even and odd numbers of carbon atoms, while *anteiso*-branched chains had entirely odd-numbered chain lengths. Straight chain alkyl and acyl groups in female ADG were all saturated, with the principal ones being C18:0 and C20:0 (alkyl groups) and C16:0 (fatty acids).

The primary endocrine control of Harderian ADG appeared to be due to androgens. In the present study, conversions from male type to female type and *vice versa* were demonstrated by castration of males and testosterone treatment of females, respectively. Castrated males had a female pattern on TLC and showed branched chain alkyl and acyl groups. Amongst the straight chain alkyl groups, C18:0 and C20:0 predominated, and of the straight chain fatty acids, C16:0 was in greatest abundance. Female hamsters treated with exogenous testosterone, on the other hand, showed male TLC patterns and entirely straight chain alkyl and acyl groups; C18:0 and C19:0 were the main alkyl groups and C15:0, C14:0 were the main fatty acids.

Androgens appear to be primarily responsible for dimorphic features of the Harderian gland in golden hamsters. Thus, cell types, organelles, porphyrin concentrations, and *N*-acetyltransferase activity are all regulated by androgens; castration transforms these features in males to the female pattern and testosterone has the opposite effect in females (*10, 16-21*).

Harderian glands of golden hamsters of both sexes appear to respond to androgen in a manner similar to accessory sex glands. In accessory sex glands, testosterone is converted by the action of 5α-reductase to 5α-dihydrotestosterone (DHT); DHT binds to the androgen receptor and stimulates androgenic characteristics by its effect on the genome. Harderian glands in both sexes of golden hamsters have 5α-reductase activity and androgen receptors (*22-25*), though Stankov and his colleagues (*26*) suggested from kinetic studies that testosterone (and not DHT) might be the active androgen in this gland. In addition, a 5α-reductase antagonist did not mimic the effects of castration on porphyrin concentrations (*27*).

Branched chain alkyl and acyl groups were present only in a low testosterone environment. Seyama *et al.* (*9*) suggested that the enzymes isovaleryl-CoA dehydrogenase (which catabolized the branched chain amino acid leucine) and 2-methyl-branched chain acyl-CoA dehydrogenase (which catabolized isoleucine and valine) were inactive in the absence of testosterone. In these circumstances, isovaleryl-CoA, 2-methylbutyryl-CoA, and isobutyryl-CoA accumulate, and these act as primers for the synthesis of odd-numbered *iso*-branched, odd-numbered *anteiso*-branched, and even-numbered *iso*-branched fatty acids, respectively. We are continuing studies addressing this hypothesis.

Odd chain-length fatty acids, particularly C15:0, are also characteristic of ADGs in a testosterone-rich environment. Odd chain-length fatty acids are synthesized when propionyl-CoA is a primer for fatty acid synthesis (*28*). This propionyl-CoA may also be a by-product of valine or

isoleucine catabolism or may originate elsewhere; elucidation of this must await further studies.

During the synthesis of ADG, a fatty acid is first linked, *via* an ester bond, to dihydroxyacetone-phosphate (DHAP) to form 1-acyl-DHAP. The enzyme 1-alkyl-DHAP synthase then cleaves this ester linkage and replaces it with a long chain fatty alcohol in an ether linkage, to form 1-alkyl-DHAP. Fatty alcohols are synthesized from fatty acids; aldehyde dehydrogenase converts fatty acid to the corresponding aldehyde and alcohol dehydrogenase converts the aldehyde to the corresponding alcohol.

Because of limited availability of material, we were unable to investigate two aspects of hamster ADG, namely, the composition of the individual subfractions obtainable on TLC (three in males, M1, M2, M3, and two in females, F1, F2), and the short chain fatty acids present at position 3 of some of these subfractions. In subfraction M3 of male ADGs, propionic, hexanoic, and octanoic acids are present, and in subfraction F2 of female ADGs, isovaleric and 2-methylbutyric acids are present (*9*). It would be interesting to know whether androgens influence these features.

Thus, androgens are essential to the control of the composition of ADGs in the golden hamster Harderian gland. However, reduction in serum androgen levels in male hamsters by a natural means (*i.e.*, short photoperiods) did not mimic castration in leading to a female pattern of lipid droplet sizes (*13*). The effects of photoperiod on the composition of golden hamster ADG will be the subject of a further communication.

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