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RESEARCH LETTER

Goat milk skin products may cause the development of goat milk allergy

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JFD and JD were responsible for the design of the study. GAM and SSZ were responsible for immunoblot laboratory work. The first draft of the manuscript was developed by JFD, GAM, JWC, SSC, SSZ, JG, KS, CS and JD, and all co-authors contributed to the finalization of the manuscript

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In the past 5 years, JAD has received honoraria for educational presentations from Astra-Zeneca, GSK, Novartis, Alphapharm, Shire, CSL. She has served on advisory boards: Sanofi-Aventis, Novartis, GSK, Astra-Zeneca, Shire, Immunosis and CSL. She has undertaken contracted or investigator-initiated research on behalf of: GSK, Novartis, Immunosis, AstraZeneca, Sanofi-Aventis, Grifols, CSL, BioCryst & Equilium. She has a personal superannuation shareholding in CSL.

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KEY MESSAGES

- We identified seven adults with new-onset IgE-mediated allergy to orally-ingested goat milk products
- Patients used goat milk skincare products prior to developing allergy, and six had atopic dermatitis
- Competitive inhibition experiments suggested the skincare products led to transcutaneous sensitisation and goat milk allergy

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TEXT

To the Editor,

Milk allergy is one of the most common food allergies in children, but usually resolves in the first years of life.[1] Adult-onset milk allergy is rare. In patients with inflammatory skin conditions such as atopic dermatitis, associations between the use of food allergen-containing skin products and systemic sensitization to that foodstuff has been demonstrated for several foods. These include peanut,[2,3] oat, cochineal and buckwheat.[4] There are four cases reported of individuals who developed goat milk allergy following use of topical goat milk skin products for managing atopic dermatitis.[5–7] This is of concern, as food-containing skin products are commonly promoted as a safer and more 'natural' way of managing a variety of skin conditions. In Australia, these are widely available for unprescribed purchase in pharmacies and supermarkets.

Within our centre, we noted an increased number of patients describing systemic reactions to goat milk or cheese ingestion and preceding use of topical goat milk products for managing inflammatory dermatopathies. Here, we present a case series of 7 goat milk allergic individuals, describe their clinical features and present laboratory findings that support the origin of their sensitisation as topical exposure to goat milk products.

Seven patients were identified using a retrospective audit of all positive (≥0.35 kUA/L) goat and/or sheep milk specific immunoglobulin E (sIgE) results performed by the Royal Melbourne Hospital Pathology Department between 2016 and 2019. A chart review of the patients' electronic medical records was undertaken and patients were invited to attend for further skin testing and blood sample collection. Cow's milk and rye grass pollen allergic controls were also evaluated. The study was approved by the Melbourne Health Human Research Ethics Committee (HREC 2018.099), and all patients provided written informed consent. Patients underwent skin prick testing to commercial goat's milk extract (ALK Abello) and prick-prick testing with sheep's milk yoghurt, camel milk, buffalo milk mozzarella and cow's milk. Fresh goat milk prick-prick testing was excluded for safety reasons. Patients were also tested for serum sIgE to cow's, goat and sheep milk, cow's milk casein and β-lactoglobulin (BLG) using ImmunoCAP platform (Phadia; Uppsala, Sweden). Serum was also stored at -80°C prior to immunoblot studies. Commercially available goat cheese samples were resolved via SDS-PAGE with standard immunoblotting methods used to measure IgE binding.

Patient case summaries are shown in Table 1. All seven individuals had symptoms consistent with IgE-mediated reaction to ingested goat or sheep milk or cheese products. All had a history of using topical goat milk products in self-management of inflammatory skin conditions prior to onset of their goat milk allergy. Six patients had atopic dermatitis and five had allergic rhinitis.

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All patients were skin prick test positive to goat milk extract and sheep milk yoghurt (Table 1). Three patients were skin test positive to only goat and sheep milk and four to at least one other milk product from buffalo mozzarella, cow's milk or camel milk.

Specific IgE findings mirrored the skin prick test results (Table 1). All patients were sIgE positive to both goat and sheep milk, with a range of 4.79-37.1 kUA/L and 0.83->100 kUA/L respectively. Three patients had detectable cow's milk sIgE (>0.1 kUA/L), which correlated with casein sIgE positivity. Only one of these three had clinical cow's milk allergy.

Goat or cow's milk soft cheese was resolved by SDS-PAGE, transferred to nitrocellulose, exposed to patient serum (1/20 dilution) and then probed with an anti-IgE-HRP (Themo Fisher, Scoresby, Victoria, Australia). Across the cohort, numerous immunoreactive bands were observed with likely multiple casein proteins (*ca.* 30 kDa) being particularly pronounced (Figure 1A). The goat milk allergic patients showed little cross-reactivity to cow's milk (patients 1 and 2, lane 3) although immunoreactivity to a lower molecular weight protein (*ca.* 15 kDa) was seen, possibly representing binding to BLG or α-lactalbumin (ALA). A recombinant human IgE specific for the hapten 4-Hydroxy-3-iodo-5-nitrophenylacetyl (NIP) (Figure 1A; lane 4) was used as a control protein throughout to ensure the efficiency of IgE detection. Cow's milk allergic patient sera, as expected, showed strong immunoreactivity to cow's milk proteins (Figure 1A; lane 3).

To test for commonality between IgE for goat cheese proteins and soap containing goat milk, competition studies were established. In these experiments, patient sera were pre-incubated with commercially available goat milk containing soaps or control soaps (0.5 mg/ml) prior to exposure to the cheese proteins on the blots. Pre-incubation with the goat milk containing soap, but not the control soap, greatly reduced sIgE binding (Figure 1Bi; representative blots shown for Patient 2). Goat milk lotion was also used by a number of our allergic cohort and could thus potentially act as the sensitising agent. Competition studies showed essentially the same findings to soap (data not shown), indicating cross-reactivity between the skincare products and patient sIgE.

To exclude inhibition of sIgE immunoreactivity as a non-specific effect of incubating serum with soap, rye grass pollen extract (RGPE) or NIP conjugated to bovine serum albumin (NIP-BSA) (Biosearch Technologies, Novato, California) were resolved and the effects of the soaps on the binding of serum IgE from a grass pollen allergic control and JW8-IgE analysed. Pre-treatment with either soap had no effect on sIgE binding to RGPE (Figure 1Bii), and a higher concentration of soap (2.5 mg/ml) caused only a small reduction inJW8-sIgE binding to NIP-BSA (Figure 1Biii)).

This case series of seven adults provides evidence for a relationship between the use of goat milk containing treatments for inflammatory skin conditions and subsequent development of new-onset severe goat milk allergy. This strengthens a series of prior case reports on this association.[5–7] It could be postulated that this association is confounded by a higher incidence of new-onset food allergy in

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atopic individuals. However, our immunoblotting studies demonstrate cross-reactivity of patient IgE to goat milk and goat milk-derived skincare products, implying a causal association. Our data also suggests that tolerance to cow's milk in this group is found in the majority, which contrasts with primary cow's milk allergy, where most patients also react to goat milk.[8]

Sensitisation through inflamed skin has been recognised as a significant risk factor for development of clinical allergy, with evidence for a causal relationship between atopic dermatitis and the development of food allergy.[9] The most comprehensively studied association has been the connection between atopic dermatitis and development of peanut allergy in children, with two foundational studies demonstrating an association between peanut allergic children with atopic dermatitis and topical peanut exposure.[2,3]

Competitive IgE binding immunoblot studies undertaken with patient serum and appropriate patient and allergen controls demonstrate significant reduction in IgE binding to goat's milk cheese products when serum was pre-incubated with goat's milk soap or goat's milk lotion. This suggests that sIgE in patient serum is binding a shared epitope found in all three products. Knowing that cutaneous sensitisation to food products does occur, these findings suggest that sensitisation to goat milk in these patients is occurring via epicutaneous exposure through the use of topical goat milk skin products.

Whilst a key limitation of this work is the small sample size, this study represents the largest cohort reported to date. Marketing of skin products derived from goat milk is extensive and targeted to patients with 'sensitive skin' who commonly have underlying inflammatory skin conditions. Our findings provide novel evidence of the origins of adult-onset milk allergy and adds to the growing body of evidence that use of food-based skincare products for inflammatory skin conditions can lead to the development of new food allergies. Taken together, the accumulating evidence that application of food products to inflamed skin promotes the development of serious food allergy has implications for regulation of the skincare industry.

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Table 1. Patient case summary and triggering events with corresponding skin prick test and specific IgE results.

Case summary	**Case 1**	**Case 2**	**Case 3**	**Case 4**	**Case 5**	**Case 6**	**Case 7**
Sex	M	F	F	F	F	M	F
Age	25	58	50	49	44	61	51
Allergic disease	Allergic rhinitis, asthma, atopic dermatitis	Atopic dermatitis	Allergic rhinitis, atopic dermatitis	Atopic dermatitis	Allergic rhinitis, atopic dermatitis	Nil	Allergic rhinitis, asthma and atopic dermatitis
Goat's milk product used	Topical use of goat's milk soap within the preceding 12 months	Topical use of goat's milk soap in the preceding five years	Topical use of goat's milk soap for a number of years prior	Topical use of goat's milk soap	Topical use of goat's milk soap over years	Topical use of goat's milk soap	Topical use of goat's milk soap
Trigger	Dip containing feta cheese	Ingestion of various cheeses	Goat's cheese on two occasions	Goat's milk ice cream	Goat's cheese	Various goat's milk cheeses, and buffalo mozzarella	Goat's and sheep's milk cheese
Clinical presentation	Anaphylaxis with throat tightness, dyspnoea,	Initial episode of throat tightness,	Recurrent episodes of urticaria and angioedema	Anaphylaxis with dyspnoea/wheeze,	Anaphylaxis with flushing, dyspnoea	Four episodes of acute urticaria and	Recurrent anaphylaxis with cutaneous

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	widespread rash and pre-syncope within 10 minutes of ingestion with resolution of symptoms after IM adrenaline	with two subsequent episodes causing widespread urticaria with dyspnoea and wheeze requiring IM adrenaline	associated with goat's milk cheese ingestion	throat tightness and urticaria within minutes of ingestion, responsive to IM adrenaline	and collapse requiring IM adrenaline. Prior to this event has had multiple mild reactions to goat's milk containing food products with throat itch for years	angioedema with dyspnoea and wheeze treated with oral antihistamines and corticosteroids.	(urticaria and angioedema) and respiratory features (wheeze and dyspnoea) following the ingesting of goat's milk cheese, responsive to adrenaline
Outcome	Tolerates cow's milk (graded oral challenge)	Tolerates cow's milk	No further reactions and avoids all dairy products	Tolerates cow's milk	Tolerates cow's milk	Tolerates cow's milk, avoids sheep's and goat's milk	Tolerates cow's milk, avoids goat's and sheep's milk
Skin prick test results							
Positive control	+	+	+	+	+	++	+
Negative control	-	-	-	-	-	-	-
*Fresh CM**	-	-	++	-	-	+	-
GM extract#	++	++	+++	+	++	++	++
*SM yoghurt**	++	++	+++	+	+++	+++	+++
*Camel milk**	+	+	-	-	-	+	-
*Buffalo mozzarella**	-	+	+++	-	-	++	N/A^
Specific IgE test results (kUA/L)							
Goat's Milk	6.31	12.7	17.5	4.79	30.9	37.1	9.24
Sheep's Milk	0.83	10.9	5.9	4.75	29.2	32.4	>100
Cow's Milk	<0.10	<0.10	1.62	<0.10	<0.10	1.75	0.30
Casein	<0.10	<0.10	0.72	<0.10	<0.10	1.36	0.88
Beta-lactoglobulin	<0.10	<0.10	0.17	<0.10	<0.10	<0.10	<0.10

SPT Results coded as: - <3mm, + >3mm, ++ 6mm, +++ 10mm

* Skin prick testing done via prick-to-prick testing using fresh milk products

Skin prick testing done using commercial extract

^Testing not available

Abbreviations: CM (Cow's Milk), GM (Goat's Milk), SM (Sheep's Milk)

Cow's milk control had a specific IgE value of 75.50 kUA/L

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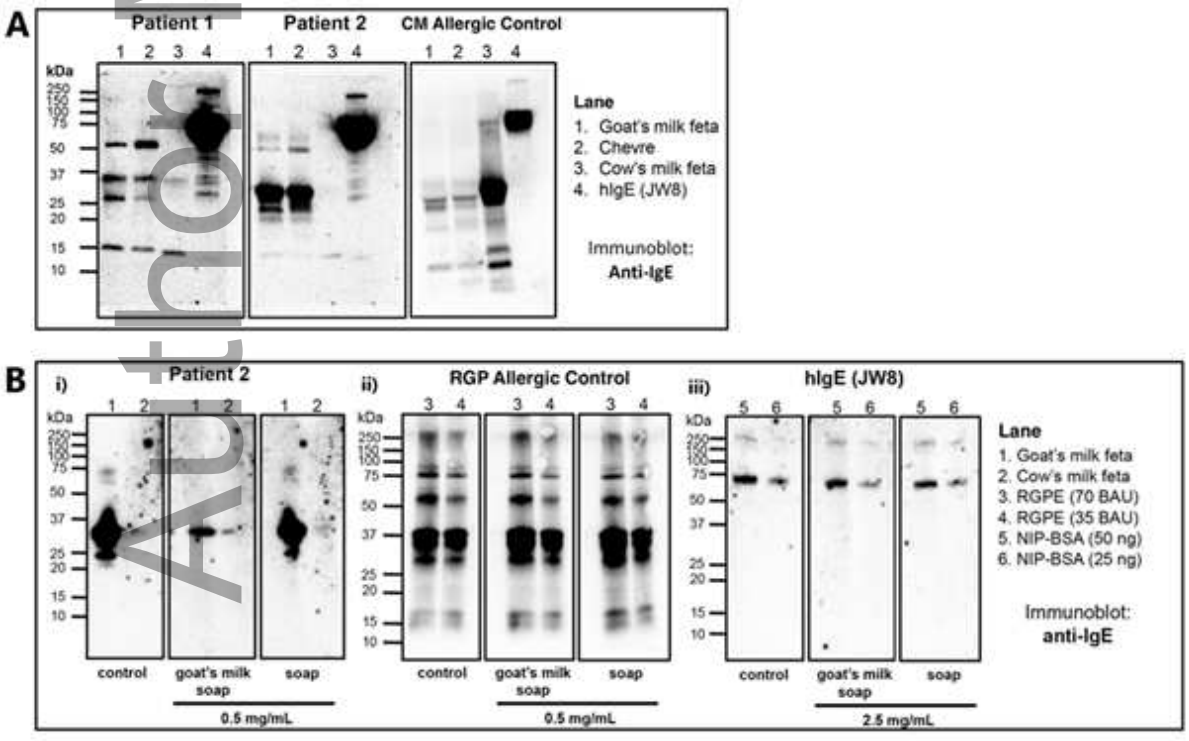

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Figure 1. Representative immunoblots showing serum IgE immunoreactivity against cheese proteins are shown in **(A)** and, competition for sIgE between goat's milk soap and resolved goat's milk cheese proteins **(B)**. Immunoblots in **(A)** show goat's milk allergic patients (patients 1/2) showed strong immunoreactivity to goat's but not cow's milk cheese proteins (lanes 1-3); control-sera showed strong immunoreactivity to cow's milk (lane 3) with recombinant human IgE (JW8) used as an immunoblot control (lane 4). Immunoblots in (**B)** show preincubation of patient serum with goat's milk containing soap, but not control soap, greatly reduced IgE binding to goat's cheese proteins **(i)**, and pre-incubation with soap did not modify RGPE specific IgE binding **(ii)** nor did it influence control IgE (JW8) binding, even at higher concentrations **(iii)**.

***Abbreviations**: kDa (kilodaltons), IgE (Immunoglobulin E), CM (Cow's Milk), RGP (Rye Grass Pollen), RGPE (RGP Extract), NIP-BSA (4-hydroxy-3-iodo-5-nitrophenylacetyl conjugated Bovine Serum Albumin)*

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Trigger	Dip containing feta cheese	Ingestion of various cheeses	Goat's cheese on two occasions	Goat's milk ice cream	Goat's cheese	Various goat's milk cheeses, and buffalo mozzarella	Goat's and sheep's milk cheese
Clinical presentation	Anaphylaxis with throat tightness, dyspnoea, widespread rash and pre-syncope within 10 minutes of ingestion with resolution of symptoms after IM adrenaline	Initial episode of throat tightness, with two subsequent episodes causing widespread urticaria with dyspnoea and wheeze requiring IM adrenaline	Recurrent episodes of urticaria and angioedema associated with goat's milk cheese ingestion	Anaphylaxis with dyspnoea/wheeze, throat tightness and urticaria within minutes of ingestion, responsive to IM adrenaline	Anaphylaxis with flushing, dyspnoea and collapse requiring IM adrenaline. Prior to this event has had multiple mild reactions to goat's milk containing food products with throat itch for years	Four episodes of acute urticaria and angioedema with dyspnoea and wheeze treated with oral antihistamines and corticosteroids.	Recurrent anaphylaxis with cutaneous (urticaria and angioedema) and respiratory features (wheeze and dyspnoea) following the ingesting of goat's milk cheese, responsive to adrenaline
Outcome	Tolerates cow's milk (graded oral challenge)	Tolerates cow's milk	No further reactions and avoids all dairy products	Tolerates cow's milk	Tolerates cow's milk	Tolerates cow's milk, avoids sheep's and goat's milk	Tolerates cow's milk, avoids goat's and sheep's milk
Skin prick test results							

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Positive control	+	+	+	+	+	++	+
Negative control	-	-	-	-	-	-	-
Fresh CM*	-	-	++	-	-	+	-
GM extract#	++	++	+++	+	++	++	++
SM yoghurt*	++	++	+++	+	+++	+++	+++
Camel milk*	+	+	-	-	-	+	-
Buffalo mozzarella*	-	+	+++	-	-	++	N/A^
Specific IgE test results (kUA/L)							
Goat's Milk	6.31	12.7	17.5	4.79	30.9	37.1	9.24
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Casein	<0.10	<0.10	0.72	<0.10	<0.10	1.36	0.88
Beta-lactoglobulin	<0.10	<0.10	0.17	<0.10	<0.10	<0.10	<0.10

SPT Results coded as: - <3mm, + >3mm, ++ 6mm, +++ 10mm

* Skin prick testing done via prick-to-prick testing using fresh milk products

Skin prick testing done using commercial extract

^Testing not available

Abbreviations: CM (Cow's Milk), GM (Goat's Milk), SM (Sheep's Milk)

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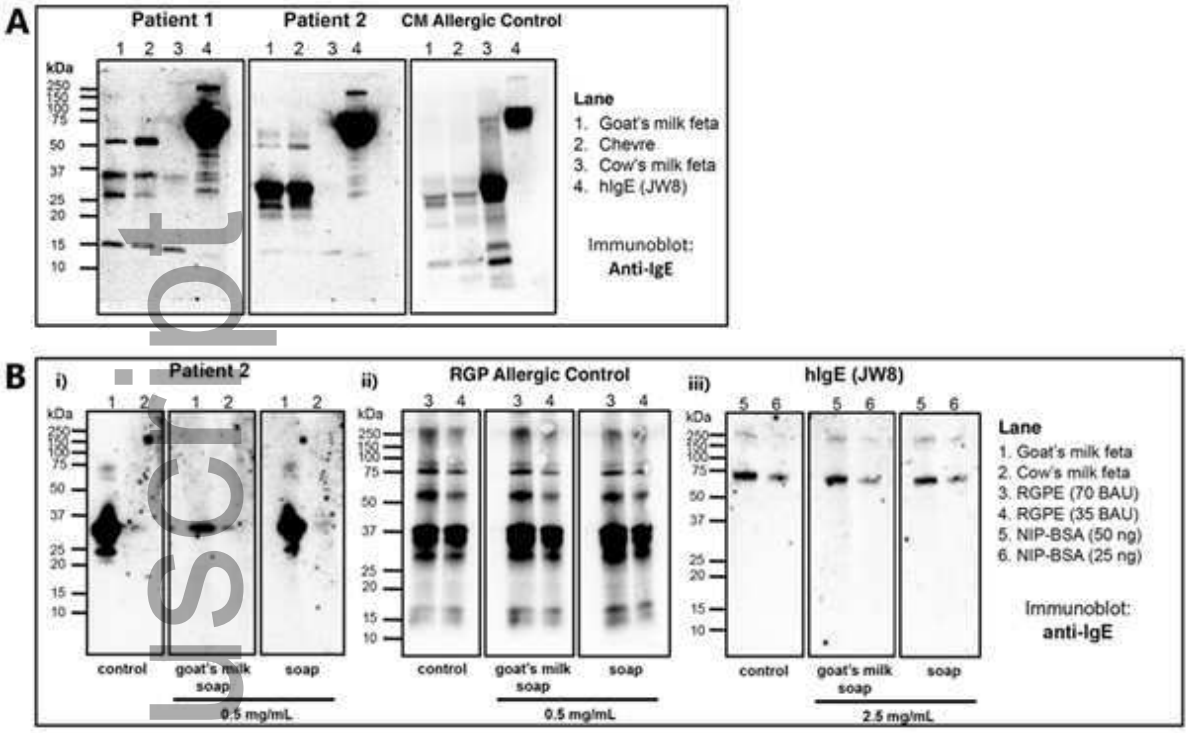

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