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ORIGINAL ARTICLE

Effects of topical corticosteroid versus tacrolimus on insulin sensitivity and bone homeostasis in adults with atopic dermatitis—A randomized controlled study

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Abstract

Introduction: Topical corticosteroids (TCS), used to treat atopic dermatitis (AD), have been associated with type 2 diabetes and osteoporosis in epidemiological studies, possibly explained by systemic absorption.

Objectives: We examined whether intensive daily whole-body TCS treatment over 2 weeks followed by twice weekly application for 4 weeks could elicit insulin resistance and increase bone resorption in adults with AD.

Methods: A randomized parallel-group double-blind double-dummy non-corticosteroid-based active comparator study design was completed in Copenhagen, Denmark.

Abbreviations: AD, atopic dermatitis; AUC, area under the curve; CTX, C-terminal telopeptide of type I collagen; NMF, natural moisturizing factor; P1NP, N-terminal propeptide of type I procollagen; Ra/Rd, rate of glucose appearance/disappearance; TCS, topical corticosteroids.

Trial registration: Clinical trials NCT04114097.

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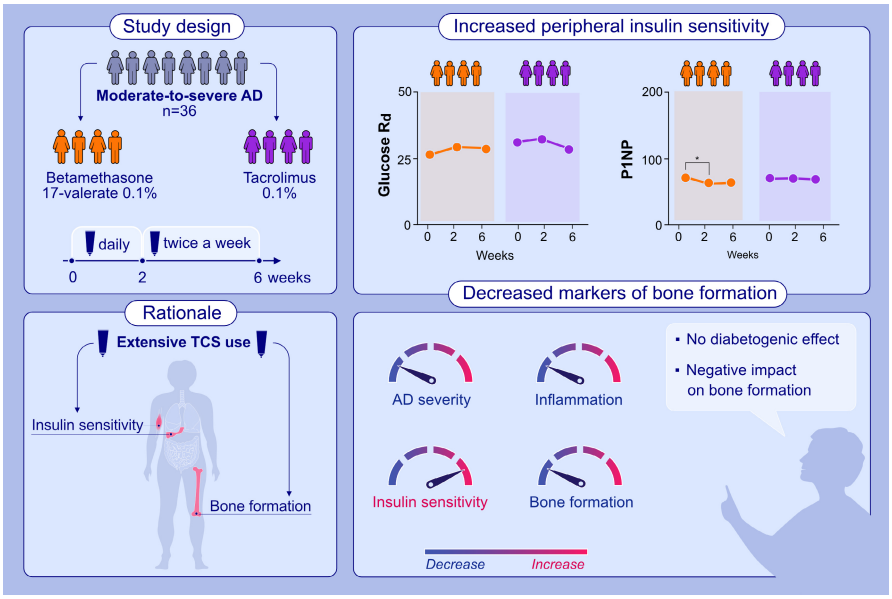
Thirty-six non-obese, non-diabetic adults with moderate-to-severe AD were randomized to whole-body treatment with betamethasone 17-valerate 0.1% plus a vehicle once daily or tacrolimus 0.1% twice daily after washout. Insulin sensitivity assessed by the hyperinsulinemic-euglycemic clamp combined with tracer infusions and biomarkers of bone formation (P1NP) and resorption (CTX) were evaluated at baseline, after 2 weeks of daily treatment and after further 4 weeks of twice-weekly maintenance treatment.

Results: AD severity improved with both treatments and systemic inflammation was reduced. After 2 weeks, we observed similar increase in peripheral insulin sensitivity with use of betamethasone ($n = 18$) and tacrolimus ($n = 18$). Bone resorption biomarker, CTX, was unchanged, while bone formation marker, P1NP, decreased after betamethasone treatment after both 2 and 6 weeks but remained unchanged in the tacrolimus arm.

Conclusions: Whole-body treatment with TCS leads to systemic exposure but appears not to compromise glucose metabolism during short-term use, which may be a result of reduced systemic inflammatory activity. The negative impact on bone formation could be regarded an adverse effect of TCS.

KEYWORDS
atopic dermatitis, calcineurin inhibitor, corticosteroid, osteoporosis, type 2 diabetes



GRAPHICAL ABSTRACT

This study examined whether intensive daily whole-body TCS treatment over 2 weeks followed by twice weekly application for 4 weeks could elicit insulin resistance and increase bone resorption in adults with AD. Insulin sensitivity increased 12.6% after 2 weeks of betamethasone and 6.40% after 6 weeks. A slight increase in insulin sensitivity was found after 2 weeks of tacrolimus treatment. P1NP decreased significantly with 10.7% in the betamethasone group after 2 weeks. AD severity improved with both treatments and systemic inflammation was reduced.

1 | INTRODUCTION

Prolonged treatment with oral corticosteroids increases the risk of type 2 diabetes and osteoporosis, and even short-term exposure reduces insulin sensitivity and alters osteoblastic function.[1–3] Inhaled corticosteroids are also associated with decreased bone density in adults with asthma and chronic obstructive pulmonary disease.[4,5]

Topical corticosteroids (TCS) used to treat inflammatory skin diseases could be associated with similar systemic endocrine effects since the molecule is small and may be absorbed after application.[6,7] However, the risk of systemic endocrine side effects when TCSs are used as recommended has been considered negligible,[8] although one treatment of 20–30 g 0.05% clobetasol propionate can cause adrenal suppression[9] and Cushing syndrome has been reported in

several case reports after clobetasol propionate use.[10] The Summary of Product Characteristics of TCSs states that the drug may cause complications such as hyperglycemia and loss of bone mineral density.[11] Recent Danish and British registry studies of adults found that use of potent and very potent TCSs was associated with a significantly increased risk of type 2 diabetes,[12] osteoporosis, and major osteoporotic fracture in dose-dependent manners.[13]

Patients with atopic dermatitis (AD), a common chronic inflammatory skin disease, have skin barrier impairment, continuously apply TCSs[14] and have an increased risk of type 2 diabetes and osteoporosis.[15–17] Therefore, our hypothesis was that patients with AD would have a higher systemic absorption of TCSs and hence a higher risk of developing systemic endocrine adverse effects such as insulin resistance and increased bone turnover during treatment. In this study, we therefore investigated whether intensive use of TCS could lead to increased systemic exposure and elicit insulin resistance and impact bone turnover compared to a topical calcineurin inhibitor (a non-corticosteroid anti-inflammatory drug).

2 | METHODS

2.1 | Study design

In this randomized, two-arm, active comparator, double-blind clinical trial, the primary endpoint was change in insulin sensitivity in adults with AD during treatment with ointment containing betamethasone 17-valerate 0.1% (a corticosteroid) compared to tacrolimus 0.1% (a calcineurin inhibitor with comparable effectiveness[18]). Secondary endpoints included change in markers of bone resorption and formation, endogenous glucose production, lipolysis, serum/plasma concentrations of insulin, C-peptide and glucagon, pancreatic β-cell function, liver status, body composition, level of physical activity, AD severity, systemic corticosteroid and calcineurin inhibitor exposure, skin barrier status, cutaneous and systemic inflammation. Medication was packed, labeled, blinded, randomized, and BMI-matched (over/under 25 kg/m^2) by Glostrup Pharmacy, Denmark. Appendix S1 contains details on randomization, protocol approval, and registration.

2.2 | Study participants and screening

Inclusion criteria were AD according to the Hanifin and Rajka Criteria,[19] AD duration >3 years, age 18–75 years, body mass index (BMI) <30 kg/m^2 and glycated hemoglobin (HbA1c) <42 mmoL/moL. Exclusion criteria were systemic AD treatments within 4 weeks before study start, (pre)diabetes, other chronic inflammatory diseases except rhinitis and asthma, smoking >10 cigarettes per day, alcohol or drug abuse, pregnancy, breastfeeding and treatment with drugs affecting glucose metabolism (e.g., systemic corticosteroids and other anti-inflammatory drugs). Daily continuous use of inhalers, nose spray, and eyedrops containing corticosteroids were allowed, but changes in medication were not allowed 4 weeks before and throughout the study. At a screening visit, medical history was obtained, physical examination was completed, and blood samples analyzed.

2.3 | Treatment regimen (Figure 1)

Prior to the first of three visits, patients endured a 2-week washout, where topical anti-inflammatory and ultraviolet treatments were discontinued. After the first visit, patients applied whole-body treatment (except face, axillae, and genitals) using a 30 g-tube for each treatment dosed by the fingertip unit (ointment dispensed on the index fingertip (~0.5 g) covers ~2% of the body surface).[20] Tacrolimus 0.1% was applied twice daily and betamethasone 17-valerate 0.1% once daily plus a vehicle once daily to maintain blinding. Following AD treatment guidelines,[8,21] patients applied treatment daily for 2 weeks between first and second visit and twice weekly for 4 weeks between second and third visit as maintenance treatment.

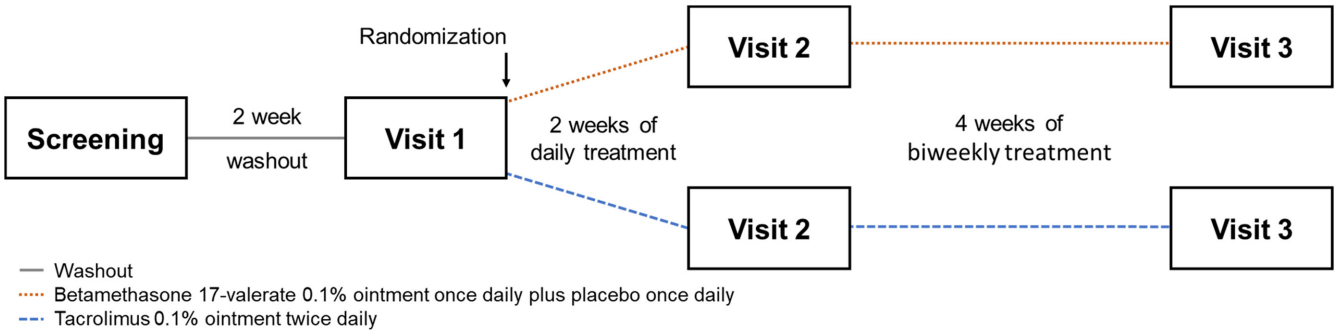


FIGURE 1 Overview of study design. After attending a screening visit, participants went through a wash-out period of 2 weeks without any topical or systemic anti-inflammatory treatment. At the first visit, participants were randomized to apply whole-body treatment with betamethasone 17-valerate 0.1% ointment once daily plus topical vehicle, or tacrolimus 0.1% ointment twice daily for 2 weeks. At the second visit, treatment was reduced to applications twice daily (betamethasone + vehicle vs. tacrolimus), but only on 2 weekdays for 4 weeks. Treatment was stopped at the third study visit.

2.4 | Procedures

For procedure details, see Appendix S1. Body composition was determined using a medical body composition analyzer, and liver stiffness and steatosis were evaluated by a transient ultrasonographic elastography. Physical activity was assessed by the short International Physical Activity Questionnaire (IPAQ) and expressed numerically by metabolic equivalents (METs).[22]

2.4.1 | Hyperinsulinemic-euglycemic clamp

A cannula was inserted in an antecubital vein in each arm, one for infusions and the other for blood sampling. After drawing fasting blood samples, [6,6-D2]-glucose and [1,1,2,3,3-D5] glycerol tracers were infused. Steady state was assumed after 90–120 min (basal steady state period). Afterwards, a 2-hour continuous infusion of insulin was initiated at 40 mU/m^2/min. To maintain euglycemia (~5 mmoL/L), the rate of a 20% glucose infusion was adjusted based on bedside plasma glucose measurements at 5-minute intervals. Clamp steady state was assumed the last 30 min. Blood samples were drawn every 15–30 min. During basal and clamp steady state, indirect calorimetry was performed to calculate rates of glucose and lipid oxidation. After the clamp, 5 g of L-arginine was infused to measure pancreatic α- and β-cell secretory capacities.

2.4.2 | Blood and tape sample analysis

Samples were frozen until batch analysis after study completion. Blood samples were analyzed for glucose and glycerol tracer enrichment, glucagon, insulin, C-peptide, metabolites of betamethasone 17-valerate or tacrolimus, markers of bone metabolism (N-terminal propeptide of type I procollagen (P1NP) and C-terminal telopeptide of type I collagen (CTX)), thymus- and activation-regulated chemokine (TARC), and interleukin (IL)-6. Skin tape samples from lesional and/or non-lesional AD skin were analyzed for TARC, IL-6 and natural moisturizing factor (NMF, a marker of skin barrier function).

2.5 | Calculations and statistical analyses

During basal and clamp steady state, rate of appearance (R_a) and disappearance (R_d) for both glucose and glycerol were calculated by Steele non-steady state equation[23] modified for stable isotope tracers as previously described.[24–26] Insulin sensitivity was measured as R_d for glucose during the clamp steady state. Insulin sensitivity was also measured from clamp steady state as the amount of infused glucose necessary to compensate for the hyperinsulinemia and expressed as a metabolizable glucose value (*M*-value).[27] A 20% difference in insulin sensitivity for betamethasone compared to tacrolimus was considered a clinically relevant difference. To detect this with 80% power at a significance level of 5%, we included 18 in each group. Changes in outcome between study days (baseline, week 2, week 6) and estimated treatment difference (ETD) were analyzed using a constrained linear mixed model[28,29] with inherent baseline adjustment. See Appendix S3 for a detailed explanation and other calculations.

3 | RESULTS

3.1 | Demographics and clinical data

Among 49 adults screened for eligibility, 36 were enrolled in the study (See Appendix S2 for CONSORT flow diagram). The two groups were overall comparable regarding age, gender, BMI, HbA1c, AD duration, and eczema severity (Table 1). More patients were filaggrin gene mutation carriers in the betamethasone group than in the tacrolimus group. There was one dropout in the tacrolimus group after 2 weeks of treatment due to non-response to treatment and severe AD. Thus, at baseline and after 2 weeks, data from 18 patients were analyzed in each group, and after 6 weeks of treatment, data from 17 and 18 patients were analyzed in the tacrolimus and betamethasone group, respectively.

3.2 | Increase in peripheral insulin sensitivity following topical treatment

Insulin sensitivity (R_d glucose) increased 12.6% ($p = .079$) after 2 weeks of betamethasone and 6.40% ($p = .377$) after 6 weeks. A slight increase in insulin sensitivity of 4.1% ($p = .562$) was found after 2 weeks of tacrolimus treatment. Comparing the treatments, we found no significant difference at week 2 ($p = .397$) or week 6 ($p = .331$, Figure 2A and Tables 2 and 3), and therefore not reaching a 20% clinically relevant difference. Insulin sensitivity calculated as *M*-value increased significantly in the betamethasone group by 15.9% ($p = .039$) after 2 weeks of treatment and by 18.8% ($p = 0.026$) after 6 weeks but without a difference to tacrolimus (Tables 2 and 3). Calculated from the indirect calorimetry, glucose oxidation remained stable, while non-oxidative glucose metabolism tended to increase after 2 weeks in both groups (Tables S1 and S2). Insulin resistance assessed by Homeostasis Model Assessment 2 (HOMA2-IR) remained the same (Tables 2 and 3).

3.3 | Decrease in endogenous glucose production and whole-body lipolysis

Endogenous glucose production tended to decrease in the betamethasone group, but not significantly compared to tacrolimus (Tables 2 and 3). Lipolysis tended to decrease after 2 weeks of betamethasone and significantly decreased after 6 weeks, but with no difference to tacrolimus (Tables 2 and 3). During the clamp, endogenous glucose production was almost 0 with a tending decrease after both treatments, indicating a lower hepatic glucose production during insulin-stimulation and hence indirectly an increased hepatic insulin sensitivity. Further, lipolysis during insulin-stimulation

TABLE 1 Participant baseline characteristics.

	Betamethasone 0.1% ointment	Tacrolimus 0.1% ointment
	n = 18	*n* = 18
Demographics		
Age in years	26 (23–37)	23 (22–28)
Female gender	9 (50.0)	10 (55.6)
Currently smoking <10 cigarettes per day	1 (5.6)	1 (5.6)
Ethnicity		
Caucasian	17 (94.4)	17 (94.4)
Mixed African and Caucasian	1 (5.6)	0
Middle Eastern/Asian	0	1 (5.6)
Clinical and biochemical data		
Body mass index (kg/m^2)	23.0 ± 3.0	23.7 ± 2.2
Glycated hemoglobin (HbA1c, mmol/mol)	30.9 ± 2.0	31.2 ± 1.9
Filaggrin gene mutation status		
Homozygous	3 (16.7)	1 (5.6)
Heterozygous	6 (33.3)	4 (22.2)
Wildtype	9 (50.0)	13 (72.2)
Atopic dermatitis		
Disease duration, years	25 (21–34)	22 (20–26)
EASI score	16.5 ± 13.7	16.3 ± 10.0
Previous systemic treatment of atopic dermatitis	1 (5.6)	1 (5.6)
Allergies		
Positive serum IgE against ≥1 aeroallergen(s)[a]	13 (72.2)	13 (72.2)
Positive serum IgE against ≥1 animal epithelia allergen(s)[b]	7 (38.9)	12 (66.7)
Total IgE (×10^3 IU/L)	167 (37–901)	627 (206–1520)
Asthma		
Previous	3 (16.7)	4 (22.2)
Current, all	3 (16.7)	4 (22.2)
Current, daily treatment with inhalation corticosteroid	2 (11.1)	2 (11.1)

Note: Normally distributed data are presented in mean ± SD, non-normally distributed data in median and interquartile range and categorical variables in numbers and percentages.

Abbreviations: AD, atopic dermatitis; EASI, Eczema Area and Severity Index; IgE, Immunoglobulin E.

[a]Aeroallergens: dust mites (dermatophagoides pteronyssinus and farinae), fungi (alternaria alternata and Cladosporium herbarum), pollen (birch, timothy grass, mugwort). Positive values >0.35.

[b]Animal epithelia allergens: dog, cat, horse. Positive values >0.35.

decreased significantly after 6 weeks of betamethasone, indicating an increased insulin sensitivity in adipose tissue, but not significant compared to tacrolimus (Tables 2 and 3). From indirect calorimetry, no significant change was found in glucose or fat oxidation (Tables S1 and S2).

3.4 | Glucose, insulin, C-peptide, and glucagon remained stable during fasting and clamp

Glucose levels at fasting conditions and during the clamp were similar in both groups at all visits (Figure 3A and Tables S1 and S2). Insulin, C-peptide, and glucagon levels are presented in Figure 3B–D and fasting and area under the curve (AUC) values in Tables S1 and S2. No differences were found in fasting or AUC values for C-peptide and glucagon. Fasting insulin levels were similar at all visits in both groups, but during the clamp, steady state AUC had increased 9.1% at week 6 in the betamethasone group with a borderline significant difference to tacrolimus of 10.3%.

3.5 | Pancreatic β-cell function remained unchanged

Glucose, C-peptide, and glucagon levels during the arginine stimulation test are presented in Figure S1A–C and AUC in Tables S1 and S2. No significant difference was found in glucose AUC and C-peptide baseline-subtracted AUC (bsAUC). However, in the betamethasone group, glucagon bsAUC increased with 28.8% after 6 weeks of treatment without a difference to tacrolimus.

3.6 | Body composition, physical activity, and liver status remained unchanged

BMI, body composition, and physical activity remained stable. Liver plasma marker, alanine transaminase, was unchanged (Tables S1 and S2), and the treatments did not induce steatosis, however, after 2 weeks, liver stiffness tended to increase with betamethasone and decrease with tacrolimus with a significant treatment difference (Tables 2 and 3). Plasma lipids remained stable except high-density lipoprotein that increased significantly with betamethasone after 2 and 6 weeks with a significant difference to tacrolimus after 2 weeks (Tables S1 and S2).

3.7 | Markers of bone formation decreased after topical corticosteroid treatment

Circulating markers of bone formation, P1NP, and resorption, CTX, are presented in Figure 2B,C and Tables 2 and 3. CTX increased

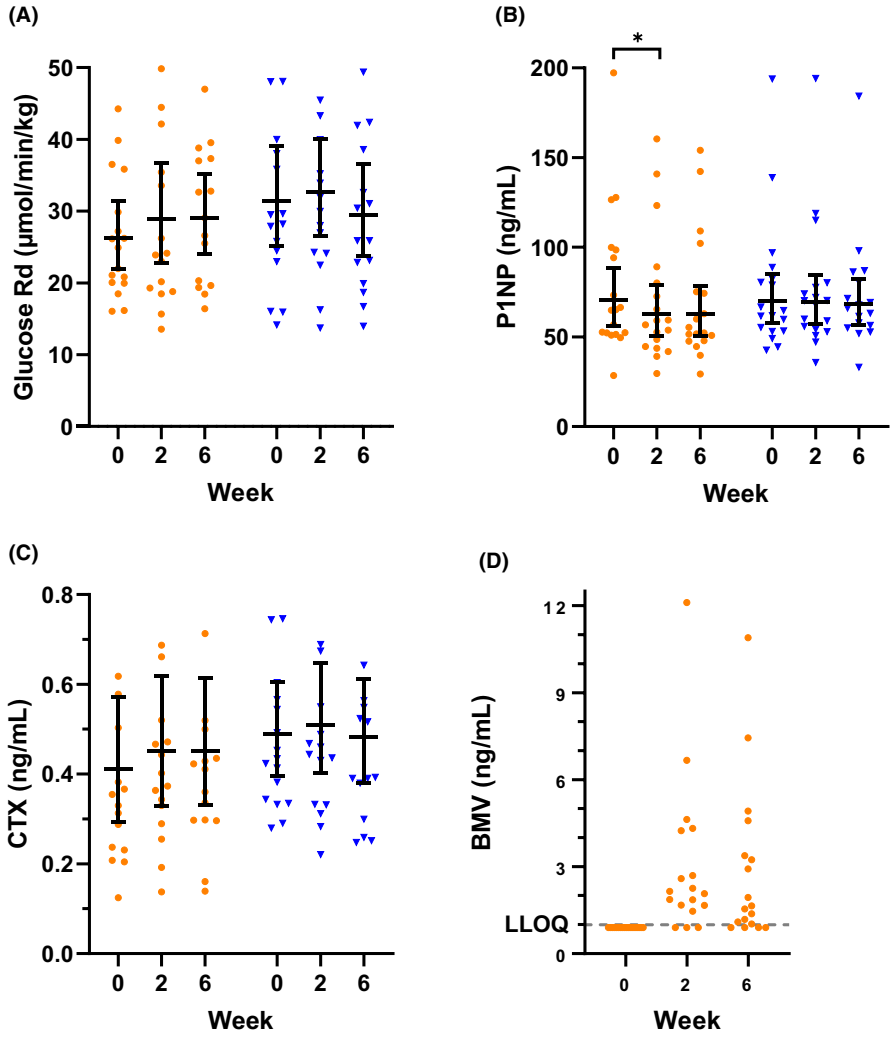


FIGURE 2 (A) Insulin sensitivity (rate of disappearance, Rd, of glucose during the hyperinsulinemic-euglycemic clamp), (B) Biomarker of bone formation (N-terminal propeptide of type I procollagen, P1NP), (C) Biomarker of bone resorption (C-terminal telopeptide of type I collagen, CTX), and D) Betamethasone 17-valerate (BMV) in plasma (with a lower limit of quantification (LLOQ) of 1 ng/mL) in patients with atopic dermatitis treated with either betamethasone 17-valerate 0.1% ointment + vehicle (orange circle) or tacrolimus 0.1% ointment (blue triangles) for an overall 6-week period. Whiskers represent geometric mean and 95% CI. Significant differences are marked with an asterisk (*).

slightly in the betamethasone arm after 2 and 6 weeks but not significantly and not compared to tacrolimus. P1NP decreased significantly with 10.7% in the betamethasone group after 2 weeks with a tending treatment difference to tacrolimus of 9.7% (Table 2). After 6 weeks, this decrease was 10.7% without a difference to tacrolimus (Table 3). Parathyroid hormone remained stable in both groups. Vitamin D slightly decreased after 2 and 6 weeks, but only significantly after 2 weeks of betamethasone with no difference to tacrolimus (Tables 2 and 3).

3.8 | Topical treatment decreased disease severity and improved quality of life

AD severity (Eczema Area and Severity Index (EASI) score[30]) and Dermatology Life Quality Index (DLQI)[31] and Patient-Oriented Eczema Measure (POEM)[32] questionnaires and visual analogue scale (VAS) for average itch intensity and average sleep disturbance are presented in Tables 2 and 3 and Tables S1 and S2, respectively, where high scores indicate severe AD or high AD burden. The severity of AD (moderate-to-severe) was based on baseline EASI score. Both treatments significantly reduced EASI to an average of "mild AD" without a significant difference between groups. Patients reported significant decreases in DLQI and POEM scores and VAS for average weekly itch after 2 and 6 weeks of treatment. Sleep disturbance significantly improved with betamethasone, but not with tacrolimus. DLQI decreased more with betamethasone than tacrolimus after 2 weeks. Treatment with betamethasone also significantly reduced itch and sleep disturbance compared to tacrolimus at weeks 2 and 6 (Tables S1 and S2).

3.9 | Cutaneous inflammation markers decreased, and marker of skin barrier function increased

After 2 weeks of treatment, cutaneous TARC, a reliable AD biomarker,[33] was reduced with both treatments with a borderline significant treatment difference of 67.6% in favor of betamethasone. Similar trends were found after 6 weeks and for cutaneous IL-6 (Tables S1 and S2). Levels of the skin barrier marker NMF from lesional and non-lesional skin are presented in Tables 2 and 3. After 2 and 6 weeks, NMF levels in lesional skin had increased by 39.7% and 0.4% with betamethasone vs 99.7% and 64% with tacrolimus

with insignificant treatment differences. In non-lesional skin, we found no significant differences for NMF between visits or treatment groups.

3.10 | Systemic inflammation markers decreased following treatment

Changes in systemic inflammatory markers are presented in Tables 2 and 3. High-sensitivity C-reactive protein (hsCRP) decreased significantly after 6 weeks of betamethasone without difference to tacrolimus. Lactate dehydrogenase significantly decreased in both groups after 2 and 6 weeks without treatment difference. Circulating eosinophils decreased significantly at weeks 2 and 6 with betamethasone and more compared to tacrolimus. Serum TARC decreased significantly in both groups at week 2 with no treatment difference. At week 6, the decrease in TARC was only significant in the tacrolimus group, but with no significant difference to betamethasone. Likewise, IL-6 decreased significantly in both groups after 2 weeks, but not after 6 weeks. Cortisol remained stable during both treatments (Tables 2 and 3).

3.11 | Betamethasone metabolites, but not tacrolimus, were detected in plasma

The mean (±SD) amount of used betamethasone and tacrolimus ointment was 17.7 ± 4.2 g and 17.3 ± 4.4 g per treatment, respectively, during daily treatment, and 19.1 ± 4.6 g and 18.3 ± 4.8 g per treatment during maintenance treatment. Plasma levels of betamethasone 17-valerate were measurable in 15 of 18 patients after 2 weeks of daily treatment, and in 14 of 18 patients after maintenance treatment (lower limit of quantification of 1 ng/mL, Figure 2A). Only one patient had undetectable levels after both 2 and 6 weeks. In the tacrolimus group, plasma tacrolimus was not measurable in any of the patients at any visit (lower limit of quantification of 1 ng/mL). The penetration dataset is available in Table S3.

3.12 | No correlation between improvements in insulin sensitivity and inflammation

We evaluated if the increase in glucose R_d was correlated with a decrease in inflammation or sleep disturbance. We found no significant correlation between percentage change in insulin sensitivity and percentage change in hsCRP, LDH, eosinophils, IL-6, TARC, or VAS of average sleep disturbance from baseline to week 2 and week 6, respectively. We also looked at the correlation between plasma levels of betamethasone 17-valerate and amount of used betamethasone, P1NP, CTX, PTH, and insulin sensitivity, and found a significant correlation between plasma levels of betamethasone 17-valerate and P1NP of −0.48 (−0.77; −0.02) after 6 weeks of treatment. This may indicate that increased penetration of betamethasone causes a stronger decrease in bone formation marker (P1NP).

4 | DISCUSSION

4.1 | Main findings

In this randomized controlled study, our hypothesis was that whole-body treatment with TCS would induce insulin resistance, whereas treatment with a topical calcineurin inhibitor would not. However, we found no clinically relevant difference between the two treatments. Treatment with betamethasone 17-valerate 0.1% ointment led to clinically meaningful reductions in signs and symptoms of AD, reduced systemic inflammation and tended to increase insulin sensitivity; in turn suggesting that reduced inflammation and/or derived improvements in symptoms of AD may have positive effects on insulin sensitivity. We also observed decreased bone formation after betamethasone treatment, which appeared to be associated with increased systemic exposure. Tacrolimus 0.1% ointment use led to overall positive effects on AD and insulin sensitivity and did not decrease bone formation.

4.2 | Interpretation of insulin sensitivity

Insulin resistance in muscle and liver is one of the initial steps in type 2 diabetes pathogenesis.[34] The hyperinsulinemic-euglycemic clamp combined with glucose tracer infusion represents the gold standard for measuring peripheral insulin sensitivity. Insulin was infused at a fixed level of 40 mU/m^2/min, which should sufficiently suppress endogenous insulin and glucose production[27,35] as we found with a calculated endogenous glucose production around 0. Surprisingly, we found a tending increase in peripheral and hepatic insulin sensitivity following betamethasone use, hence challenging the findings from recent epidemiological studies and our primary hypothesis.[12,36] In our Danish registry-based study of adults with AD, we found a correlation between TCS use and type 2 diabetes risk,[37] and a general population study showed that common *FLG* mutations, leading to skin barrier impairment, were associated with type 2 diabetes independent of present AD[15]. Further, we showed that use of TCS was associated with type 2 diabetes in Denmark and the UK[12]. Importantly, the current study examined short-term TCS exposure, and long-term continuous TCS exposure could possibly be diabetogenic.

The tending increased insulin sensitivity found in the treatment groups could be caused by a reduction in both cutaneous and system inflammation. Systemic and cutaneous IL-6 decreased in both groups, and is a biomarker known to be significantly associated with an increased risk of type 2 diabetes,[38] in turn indicating the importance of keeping AD inflammation to a minimum. In recent years, AD has increasingly been viewed as a systemic inflammatory disease.[39–41] Mild AD does not show systemic inflammation,[42] which fits well with our recent study that showed no difference in insulin sensitivity or other gluco-metabolic characteristics between patients with mild-to-moderate AD and healthy controls.[43] Other inflammatory diseases such as psoriasis and rheumatoid arthritis have also been associated with insulin resistance[2,44] and

TABLE 2 Change from baseline to week 2.

	Week 0		Week 2														
	Baseline		After 2 weeks of daily treatment														
	Pooled group		Betamethasone 0.1% ointment					Tacrolimus 0.1% ointment					Estimated treatment difference				
	Estimate at baseline	95% CI	Change from baseline		95% CI		Raw *p*-value	Adjusted *p*-value	Change from baseline		95% CI		Raw *p*-value	Adjusted *p*-value	Treatment difference		95% CI		Raw *p*-value	Adjusted *p*-value
Insulin sensitivity																				
Glucose Rd, μmoL/min/kg BW (clamp)	31.1	26.7; 35.5	3.92		−0.48; 8.32		0.079	–	1.27		−3.13; 5.67		.562	–	2.65		−3.63; 8.93		.397	–
Glucose Rd percentage change, %			12.6	%					4.08	%					7.58	%				
M-value, mg/kg/min	5.5	4.7; 6.4	15.9	%	0.8; 33.4	%	0.039*	–	8.5	%	−5.7; 24.8	%	.248	–	6.9	%	−12.3; 30.3	%	.498	–
HOMA2-IR	0.8	0.7; 0.9	−0.7	%	−9.1; 8.5	%	0.876	0.943	−3.5	%	−11.7; 5.4	%	.416	.678	3.0	%	−9.3; 16.8	%	.643	.821
Tracer calculations during basal steady state period																				
Glucose Ra (endogenous glucose production), μmoL/min/kg BW	9.43	8.78; 10.1	−0.72		−1.43; −0.02		0.044*	0.194	−0.44		−1.15; 0.26		.213	.477	−0.28		−1.20; 0.64		.536	.767
Glycerol Ra (lipolysis), μmoL/min/kg BW	3.05	2.75; 3.35	−0.39		−0.71; −0.07		0.019*	0.100	−0.16		−0.48; 0.16		.314	.565	−0.23		−0.67; 0.21		.303	.551
Tracer calculations during clamp steady state period																				
Glucose infusion rate, μmoL/min/kg BW	33.3	28.2; 38.4	5.15		−0.12; 10.4		0.055	0.223	2.97		−2.30; 8.24		.260	.519	2.18		−5.25; 9.61		.555	.783
Endogenous glucose production, μmoL/min/kg BW	−2.53	−3.94; −1.12	−1.14		−3.30; 1.01		0.289	0.542	−1.32		−3.48; 0.83		.221	.487	0.18		−2.81; 3.17		.904	.951
Glucose Ra, μmoL/min/kg BW	30.9	26.4; 35.5	4.58		0.17; 9.00		0.042*	0.190	1.21		−3.21; 5.62		.582	.793	3.38		−2.91; 9.67		.283	.538
Glycerol Ra, μmoL/min/kg BW	1.16	1.04; 1.27	0.02		−0.13; 0.16		0.836	0.925	−0.07		−0.22; 0.08		.364	.620	.08		−0.12; 0.29		.420	.680
Bone markers in serum																				
PTH, pmoL/L	4.80	4.31; 5.34	6.5	%	−4.6; 19.0	%	0.255	0.512	2.1	%	−8.6; 14.1	%	.701	.849	4.3	%	−10.7; 21.8	%	.584	.793
CTX, ng/mL	0.45	0.37; 0.54	9.5	%	−1.3; 21.4	%	0.084	0.275	4.6	%	−5.7; 15.9	%	.385	.646	4.7	%	−9.6; 21.2	%	.530	.765
P1NP, ng/mL	70.4	61.1; 81.1	−10.7	%	−17.4; −3.4	%	0.006*	0.042*	−1.1	%	−8.6; 7.0	%	.779	.892	−9.7	%	−19.2; 1.0	%	.072	.252
Vitamin D (25-OH-Vitamin D), nmoL/L	72.8	66.0; 79.6	−6.1		−9.7; −2.5		0.002*	0.015*	−3.2		−6.9; 0.4		.077	.261	−2.8		−7.8; 2.1		.250	.506

TABLE 2 (Continued)

	Week 0		Week 2																
	Baseline		After 2 weeks of daily treatment																
	Pooled group		Betamethasone 0.1% ointment					Tacrolimus 0.1% ointment					Estimated treatment difference						
	Estimate at baseline	95% CI	Change from baseline		95% CI		Raw *p*-value	Adjusted *p*-value	Change from baseline		95% CI		Raw *p*-value	Adjusted *p*-value	Treatment difference		95% CI		Raw *p*-value	Adjusted *p*-value
Hepatic transient ultrasonographic elastography (FibroScan)																			
Liver stiffness, kPa	4.0	3.7; 4.4	10.8	%	1.3; 21.3	%	0.026*	0.128	−8.7	%	−16.5; −0.1	%	.049*	.203	21.3	%	7.1; 37.5	%	.003*	.027*
Steatosis, CAP, dB/m	204	192; 216	4.3	%	−4.0; 13.4	%	0.307	0.555	−2.0	%	−9.8; 6.5	%	.632	.817	6.4	%	−4.7; 18.9	%	.261	.519
Atopic dermatitis evaluation																			
EASI score	16.4	12.4; 20.4	−13.6		−16.9; −9.8		<0.001*	<0.001*	−11.4		−14.9; −7.8		<.001*	<.001*	−2.0		−5.0; 1.0		.190	.441
Natural moisturizing factor																			
Lesional skin, mmoL/g protein	0.28	0.22; 0.37	39.7	%	−2.2; 99.5	%	0.065	0.236	99.7	%	39.2; 186.5	%	<.001*	.005*	−30.0	%	−56.7; 13.1	%	.140	.377
Non-lesional skin, mmoL/g protein	0.36	0.28; 0.45	−11.7	%	−33.7; 17.6	%	0.386	0.646	14.1	%	−14.4; 51.9	%	.360	.615	−22.6	%	−46.0; 11.0	%	.158	.399
Markers of inflammation and cortisol in blood																			
hsCRP, mg/L	1.00	0.67; 1.49	−29.7	%	−55.0; 10.0	%	0.119	0.345	−28.0	%	−54.0; 12.6	%	.145	.383	−2.3	%	−47.7; 82.5	%	.940	.969
Lactate dehydrogenase, U/L	194	176; 215	−15.5	%	−22.7; −7.7	%	<0.001*	0.005*	−11.4	%	−18.9; −3.1	%	.009*	.056	−4.7	%	−15.1; 7.1	%	.410	.671
Eosinophils, $\times 10^9$/L	0.37	0.28; 0.50	−57.1	%	−66.1; −45.6	%	<0.001*	<0.001*	−23.7	%	−39.5; −3.8	%	.024*	.121	−43.7	%	−58.9; −22.9	%	.001*	.009*
TARC/CCL17, pg/mL	1497	1172; 1913	−53.9	%	−64.4; −40.4	%	<0.001*	<0.001*	−51.1	%	−62.4; −36.5	%	<.001*	<.001*	−5.7	%	−33.2; 33.2	%	.733	.874
IL-6, pg/mL	6.3	4.7; 8.5	−27.0	%	−40.5; −10.4	%	0.004*	0.028*	−28.2	%	−41.8; −11.5	%	.003*	.024*	1.8	%	−23.8; 36.0	%	.903	.951
Cortisol, nmoL/L	309	259; 370	−7.7	%	−17.2; 2.8	%	0.139	0.377	6.2	%	−4.9; 18.7	%	.276	.534	−13.2	%	−24.4; −0.2	%	.047*	.203

Note: At baseline (Week 0), normally distributed continuous variables are reported as means with 95% confidence interval (CI) and non-normally distributed data were log-transformed before analysis and presented as geometric means with 95% CI. Change from baseline to Week 2 and estimated treatment difference are presented as absolute change (95% CI, *p*-value) for normally distributed variables and as percentage change (95% CI, *p*-value) for non-normally distributed variables. Significant *p*-values are marked with an asterisk (*).

Abbreviations: AUC, area under the curve; bsAUC, baseline-subtracted area under the curve; BW, bodyweight; CAP, Controlled Attenuation Parameter; CCL17, C-C Motif Chemokine Ligand 17; CTX, C-terminal cross-linking telopeptides of type I collagen; EASI, eczema area and severity index; HOMA2-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; *M*-value, metabolizable glucose; PTH, parathyroid hormone; P1NP, N-terminal cross-linking propeptide of type 1 procollagen; Ra, rate of appearance; Rd, rate of disappearance; TARC, thymus- and activation-regulated chemokine.

TABLE 3 Change from baseline to week 6.

	Week 0		Week 6															
	Baseline		After 2 weeks of daily treatment and 4 weeks of biweekly treatment															
	Pooled group		Betamethasone 0.1% ointment					Tacrolimus 0.1% ointment					Estimated treatment difference					
	Estimate at baseline	95% CI	Change from baseline		95% CI		Raw *p*-value	Adjusted *p*-value	Change from baseline		95% CI		Raw *p*-value	Adjusted *p*-value	Treatment difference		95% CI		Raw *p*-value	Adjusted *p*-value
Insulin sensitivity																				
Glucose Rd, μmoL/min/kg BW (clamp)	31.1	26.7; 35.5	1.99		−2.52; 6.50		.377	–	−0.96		−5.38; 3.46		.663	–	2.95		−3.13; 9.03		.331	–
Glucose Rd percentage change, %			6.40	%					−3.08	%					8.91	%				
M-value, mg/kg/min	5.5	4.7; 6.4	18.8	%	2.3; 38.0	%	.026*	–	1.9	%	−12.0; 18.2	%	.793	–	16.5	%	−5.5; 43.7	%	.148	–
HOMA2-IR	0.8	0.7; 0.9	6.9	%	−2.9; 17.7	%	.166	.403	−2.6	%	−11.7; 7.4	%	.584	.793	9.8	%	−3.8; 25.3	%	.159	.399
Tracer calculations during basal steady state period																				
Glucose Ra (endogenous glucose production), μmoL/min/kg BW	9.43	8.78; 10.1	−0.71		−1.44; 0.03		.058	.230	−0.59		−1.35; 0.16		.118	.345	−0.11		−1.12; 0.89		.818	.916
Glycerol Ra (lipolysis), μmoL/min/kg BW	3.05	2.75; 3.35	−0.53		−0.85; −0.21		.002*	.0170*	−0.33		−0.66; 0.00		.050	.204	−0.20		−0.63; 0.23		.351	.603
Tracer calculations during clamp steady state period																				
Glucose infusion rate, μmoL/min/kg BW	33.3	28.2; 38.4	5.24		−0.16; 10.7		.057	.227	1.34		−4.00; 6.67		.614	.817	3.91		−3.60; 11.4		.298	.546
Endogenous glucose production, μmoL/min/kg BW	−2.53	−3.94; −1.12	−3.14		−5.70; −0.57		.018*	.097	−2.36		−4.85; 0.14		.063	.234	−0.78		−4.26; 2.71		.652	.821
Glucose Ra, μmoL/min/kg BW	30.9	26.4; 35.5	2.44		−2.20; 7.07		.294	.546	−1.26		−5.82; 3.30		.579	.793	3.70		−2.50; 9.90		.234	.499
Glycerol Ra, μmoL/min/kg BW	1.16	1.04; 1.27	−0.16		−0.28; −0.03		.013*	.076	−0.07		−0.19; 0.04		.215	.477	−0.08		−0.24; 0.08		.297	.546

TABLE 3 (Continued)

	Week 0		Week 6															
	Baseline		After 2 weeks of daily treatment and 4 weeks of biweekly treatment															
	Pooled group		Betamethasone 0.1% ointment					Tacrolimus 0.1% ointment					Estimated treatment difference					
	Estimate at baseline	95% CI	Change from baseline		95% CI		Raw *p*-value	Adjusted *p*-value	Change from baseline		95% CI		Raw *p*-value	Adjusted *p*-value	Treatment difference		95% CI		Raw *p*-value	Adjusted *p*-value
Bone markers in serum																				
PTH, pmoL/L	4.80	4.31; 5.34	−1.1	%	−12.5; 11.7	%	.851	.933	9.6	%	−3.2; 24.1	%	.143	.381	−9.8	%	−24.2; 7.4	%	.237	.499
CTX, ng/mL	0.45	0.37; 0.54	8.5	%	−4.2; 22.9	%	.192	.443	−0.4	%	−12.2; 13.1	%	.955	.974	8.9	%	−8.7; 29.9	%	.333	.584
P1NP, ng/mL	70.4	61.1; 81.1	−10.7	%	−19.5; −1.0	%	.032*	.147	−2.2	%	−11.9; 8.7	%	.677	.831	−8.8	%	−21.0; 5.4	%	.205	.463
Vitamin D (25-OH-Vitamin D), nmoL/L	72.8	66.0; 79.6	−6.6		−13.8; 0.6		.069	.247	−5.9		−13.2; 1.5		.113	.339	−0.8		−10.7; 9.2		.879	.943
Hepatic transient ultrasonographic elastography (FibroScan)																				
Liver stiffness, kPa	4.0	3.7; 4.4	5.4	%	−4.5; 16.4	%	.288	.542	−2.4	%	−11.8; 8.0	%	.627	.817	8.0	%	−5.0; 22.9	%	.229	.499
Steatosis, CAP, dB/m	204	192; 216	1.1	%	−9.2; 12.4	%	.842	.928	3.6	%	−7.2; 15.6	%	.520	.754	−2.4	%	−15.6; 12.8	%	.732	.874
Atopic dermatitis evaluation																				
EASI score	16.4	12.4; 20.4	−13.2		−16.5; −9.9		<.001*	<.001*	−11.3		−14.6; −8.0		<.001*	<.001*	−1.9		−4.8; 0.9		.166	.403
Natural moisturizing factor																				
Lesional skin, mmol/g protein	0.28	0.22; 0.37	0.4	%	−31.0; 46.2	%	.981	.987	64.0	%	12.0; 140.2	%	.012*	.072	−38.8	%	−59.4; −7.5	%	.021*	.108
Non-lesional skin, mmoL/g protein	0.36	0.28; 0.45	−16.3	%	−39.5; 15.8	%	.275	.534	24.8	%	−10.4; 73.7	%	.184	.435	−32.9	%	−54.8; −0.4	%	.048*	.203
Markers of inflammation and cortisol in blood																				
hsCRP, mg/L	1.00	0.67; 1.49	−42.4	%	−60.5; −15.9	%	.005*	.038*	−26.1	%	−49.4; 7.9	%	.115	.341	−22.0	%	−53.2; 29.8	%	.328	.581
Lactate dehydrogenase, U/L	194	176; 215	−15.6	%	−22.1; −8.6	%	<.001*	.001*	−12.6	%	−19.4; −5.2	%	.002*	.016*	−3.5	%	−12.7; 6.7	%	.476	.723
Eosinophils, $\times 10^9$/L	0.37	0.28; 0.50	−48.3	%	−59.7; −33.7	%	<.001*	<.001*	−17.2	%	−35.6; 6.4	%	.134	0.369	−37.6	%	−55.3; −12.8	%	.008*	.049*
TARC/CCL17, pg/mL	1497	1172; 1913	−21.5	%	−39.2; 1.3	%	.062	.232	−35.2	%	−50.1; −15.7	%	.002*	.017*	21.0	%	−14.6; 71.6	%	.273	.534
IL-6, pg/mL	6.3	4.7; 8.5	−19.6	%	−33.6; −2.5	%	.027*	.131	−20.5	%	−34.9; −2.9	%	.026*	.128	1.1	%	−22.9; 32.5	%	.935	.969
Cortisol, nmoL/L	309	259; 370	−1.0	%	−16.5; 17.5	%	.910	.954	0.8	%	−15.4; 20.1	%	.924	.963	−1.8	%	−21.1; 22.3	%	.868	.943

Note: At baseline (Week 0), normally distributed continuous variables are reported as means with 95% confidence interval (CI) and non-normally distributed data were log-transformed before analysis and presented as geometric means with 95% CI. Change from baseline to Week 6 and estimated treatment difference are presented as absolute change (95% CI, *p*-value) for normally distributed variables and as percentage change (95% CI, *p*-value) for non-normally distributed variables. Significant *p*-values are marked with an asterisk (*).

Abbreviations: AUC, area under the curve; bsAUC, baseline-subtracted area under the curve; BW, bodyweight; CAP, Controlled Attenuation Parameter; CCL17, C-C Motif Chemokine Ligand 17; CTX, C-terminal cross-linking telopeptides of type I collagen; EASI, eczema area and severity index; HOMA2-IR, homeostatic model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; *M*-value, metabolizable glucose; PTH, parathyroid hormone; P1NP, N-terminal cross-linking propeptide of type 1 procollagen; Ra, rate of appearance; Rd, rate of disappearance; TARC, thymus- and activation-regulated chemokine.

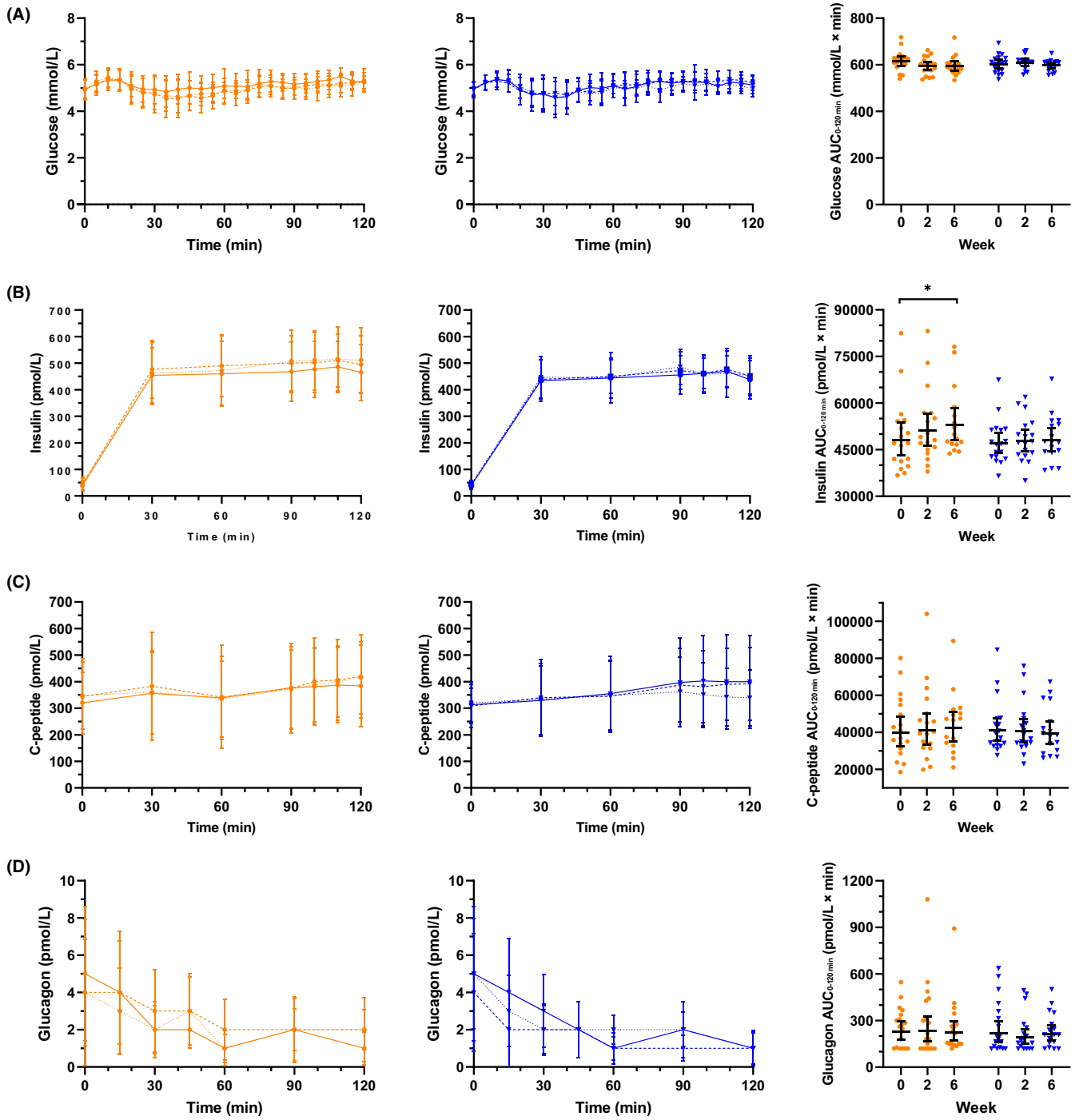


FIGURE 3 Hyperinsulinemic-euglycemic clamp in adult patients with atopic dermatitis treated with either betamethasone 17-valerate 0.1% ointment (orange circles) or tacrolimus 0.1% ointment (blue triangles). Excursions of (A) plasma glucose, (B) serum insulin, (C) serum C-peptide, and (D) plasma glucagon during the hyperinsulinemic euglycemic clamp are presented by to graphs on the left (data are presented as mean ± SD) at three different visits (visit 1 represented by a solid line, visit 2 a dashed line and visit 3 a dotted line). Grouped AUC are compared to the right (whiskers represent mean/geometric mean and 95% CI). Significant differences are marked with an asterisk (*).

development of type 2 diabetes.[45-48] Another possible influence on insulin sensitivity in patients with AD is disturbed sleep, which may negatively impact insulin sensitivity and metabolism.[49,50] However, we could not establish a correlation between improvement in sleep or inflammation and insulin sensitivity in a study with this sample size.

Type 2 diabetes is, besides insulin resistance, characterized by impaired insulin secretion from pancreatic β-cells preceded by hyperinsulinemia.[34] Hyperinsulinemia emerges from increased insulin secretion[51] and reduced insulin clearance.[52,53] Reduced clearance is seen prior to development of changes in peripheral insulin action.[54] During the clamps, identical doses of insulin were infused at each

visit; however, we found increased levels of insulin in the betamethasone group. This could partly explain the increased insulin sensitivity. We speculate that insulin clearance might have decreased during the clamps caused by betamethasone treatment. In support of this, dexamethasone reduced hepatic insulin-degrading enzyme activity in rats causing hyperinsulinemia and reduced insulin clearance.[55] Contradicting, fasting levels of insulin remained the same. We also found increased liver stiffness in the betamethasone group, but the failure rate may be high, and a study found failure range of 3%–27% for liver fibrosis staging.[56] During the arginine stimulation test, there were increased levels of glucagon in the TCS group at week 6, which is seen in type 2 diabetes and may reflect glucose and insulin resistance in α-cells.[57]

4.3 | Interpretation of bone markers

We hypothesized that bone turnover would be negatively affected by TCS treatment as corticosteroids are known to decrease bone formation by suppressing osteoblast activity and increase bone resorption by stimulating osteoclast activity.[58] Even short-term daily low-dose oral corticosteroid treatment decreases bone formation in postmenopausal women.[59] P1NP and CTX are the recommended biomarkers of bone formation and resorption,[60] respectively, and P1NP is a potential marker for early detection of osteoporosis.[61] Betamethasone decreased P1NP, which may explain the increased risk of osteoporosis seen with TCS use.[13] Treatment with corticosteroid inhalants in obstructive pulmonary disease increases the risk of fractures and osteoporosis,[4,5] and likewise, our findings indicate that systemic effects may be a risk with intense and high-dose TCS use. The decreased vitamin D in the betamethasone group should not explain the decrease of P1NP because they are not correlated.[62]

The observed effect on bone formation could be caused by increased levels of betamethasone 17-valerate in plasma as seen in our correlation analysis. We measured plasma levels about 8–26 h after application, and higher plasma concentrations could be expected in the beginning of treatment because patients had more lesional skin and presumably a higher uptake. Tacrolimus was not measurable in circulation as previously found in patients with AD, where levels did not exceed 1 ng/mL.[63] The skin barrier marker, NMF, improved during treatment with tacrolimus, whereas the positive effects of betamethasone after 6 weeks was marginal, similar to previous findings.[64]

4.4 | Limitations

Whole-body treatment for 2 weeks is indicated for patients with severe and persistent AD covering most of the body. However, we aimed to investigate the impact of maximal treatment, and even after this intense treatment, some of our patients were not completely cleared of AD, indicating that the treatment was not excessive. Lack of a placebo group may be considered a limitation; however, 8 weeks without any topical anti-inflammatory treatment would be unethical and cause many dropouts due to AD flaring.

5 | CONCLUSION

Intense short-term whole-body treatment of AD with topical betamethasone reduced disease severity, became systemically available and was associated with a reduction in systemic inflammation, improved insulin sensitivity, but reduced bone formation. Similar effects were seen with topical tacrolimus treatment, but without affecting bone formation. We found no significant difference between treatments. Our findings indicate that a reduction in skin and systemic inflammation in AD improves insulin sensitivity, in turn outweighing the possible diabetogenic effects of short-term TCS use. Putative negative effects of bone homeostasis in long-term TCS users need to be determined in future studies.

AUTHOR CONTRIBUTIONS

L.G. conceptualized, planned, and executed the study, wrote applications for funding, conducted the clinical experiments, researched the data, performed statistical analyses, and wrote the manuscript. S.K. provided multiplex analyses of inflammatory markers in skin and serum samples. I.J. provided analyses of natural moisturizing factor in skin samples. A.J.P. analyzed plasma betamethasone and tacrolimus. J.F. supervised statistical analyses. G.v.H. provided mass spectrometry analyses of isotope tracers. K.S-J., B.H., and J.J.H. provided radioimmunoassay analyses of glucagon and bone markers. H.S., L.S., and O.E.S. planned the study and wrote the manuscript. M.A.R. wrote applications for funding, planned the study, and wrote the manuscript. J.P.T. and F.K.K. conceptualized the study, wrote applications for funding, planned the study, and wrote the manuscript. All authors contributed to discussion and critically reviewed the manuscript. F.K.K., J.P.T., and L.G. are the guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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The Innovation Fund Denmark, LEO Pharma A/S, Aage Bang's Foundation.

CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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