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Review

Physiological roles of glycerol-transporting aquaporins: the aquaglyceroporins

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Abstract. A subclass of aquaporin (AQP) water channels, termed aquaglyceroporins, are also able to transport glycerol and perhaps urea and other small solutes. Although extensive data exist on the physiological roles of aquaporin-facilitated water transport, until recently the biological significance of glycerol transport by the mammalian aquaglyceroporins has been unknown. There is now compelling evidence for involvement of aquaglyceroporin-facilitated glycerol transport in skin hydration and fat cell metabolism. Mice deficient in AQP3 have dry skin, reduced skin elasticity and impaired epidermal biosynthesis. Mice lacking AQP7 manifest progressive adipocyte fat accumulation and hypertrophy. These skin and fat phenotypes are attributable to impaired glycerol transport. A potential implication of these findings is the possibility of modulation of aquaglyceroporin expression or function in the therapy of skin diseases and obesity.

Keywords. Aquaporin, AQP3, AQP7, skin, epidermis, adipocyte, transgenic mouse.

Introduction

The aquaporins (AQPs) are a family of small, hydrophobic, integral membrane proteins that are expressed widely in animals, plants, insects, amphibia, yeast and bacteria. To date, 13 mammalian AQPs have been identified (AQP 0–12). Functional studies have identified a subgroup of AQPs (AQPs 3, 7 and 9), termed aquaglyceroporins, that transport water as well as glycerol and possibly urea and other small solutes (reviewed in [1, 2]). Structural studies have begun to elucidate the molecular basis of solute transport by the aquaglyceroporins (reviewed in [3]). Based largely on phenotype analysis of AQP knockout mice, there are now extensive data on the roles of AQP-facilitated water transport in the urinary concentrating mechanism, epithelial fluid secretion, cerebral edema, neural signal transduction and cell migration (reviewed in [4]). However, until recently the physiologically important non-water transport role(s) of the aquaglyceroporins have been unknown. This review is focused on recent data implicating AQP3- and AQP7-facilitated glycerol transport in skin and adipocyte biology, with a brief discussion of AQP9.

Aquaporin-3

AQP3 was cloned initially from rat kidney [5–7], and is expressed at the basolateral membrane of epithelial cells in kidney collecting duct, airways and intestine, as well as in epidermis, urinary bladder, conjunctiva and cornea [4]. These original cloning studies and follow-up data from multiple labs have demonstrated AQP3-facilitated water and glycerol transport [8, 9]. AQP3-mediated water permeability was also reported to be pH-dependent, decreasing at low pH [10]. Initial phenotype analysis of AQP3 null mice, generated by targeted gene disruption, showed a urinary concentrating defect resulting from

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reduced water permeability in collecting duct [11]. Reduced water and glycerol permeabilities of AQP3 null mice have been demonstrated in ocular surface tissues [12], though no physiological abnormalities were found. Also, despite AQP3-facilitated osmotic water transport in large airways, AQP3 deletion in mice did not alter airway hydration or surface liquid properties [13].

Role of AQP3 as a glycerol transporter in epidermis

The most superficial layer of skin is the stratum corneum (SC), which consists of terminally differentiated keratinocytes (corneocytes) that originate from actively proliferating keratinocytes in lower epidermis (basale, spinosum and granulosum cells) and contain a lamellar lipid layer secreted from lamellar bodies (Fig. 1a) [14, 15]. Hydration of the SC is an important determinant of skin appearance and physical properties, and depends on a number of factors, including the external humidity, and its structure, lipid/protein composition, barrier properties and concentration of water-retaining osmolytes (natural moisturizing factors, NMFs) such as free amino acids, ions and other small solutes [16, 17]. Reduced SC hydration is found in aged skin, and in skin diseases including atopic dermatitis, eczema, psoriasis, senile xerosis and hereditary ichthyosis [18–22].

AQP3 is expressed in epidermal keratinocytes in rat [23, 24], mouse [25] and human [26, 27] skin. Immunocytochemistry shows AQP3 localization in plasma membranes of the basal epidermal cell layer (Fig. 1b).

Phenotype analysis of AQP3-deficient mice in a hairless genetic background generated by our lab indicated an important role of AQP3 epidermal biology. An interesting observation in AQP3-deficient epidermis was reduced SC hydration as measured by high-frequency skin conductance (Fig. 1c) [25], reduced skin elasticity, impaired reformation of the stratum corneum after removal by tape-stripping (Fig. 1d) and delayed wound healing (Fig. 1e) [28].

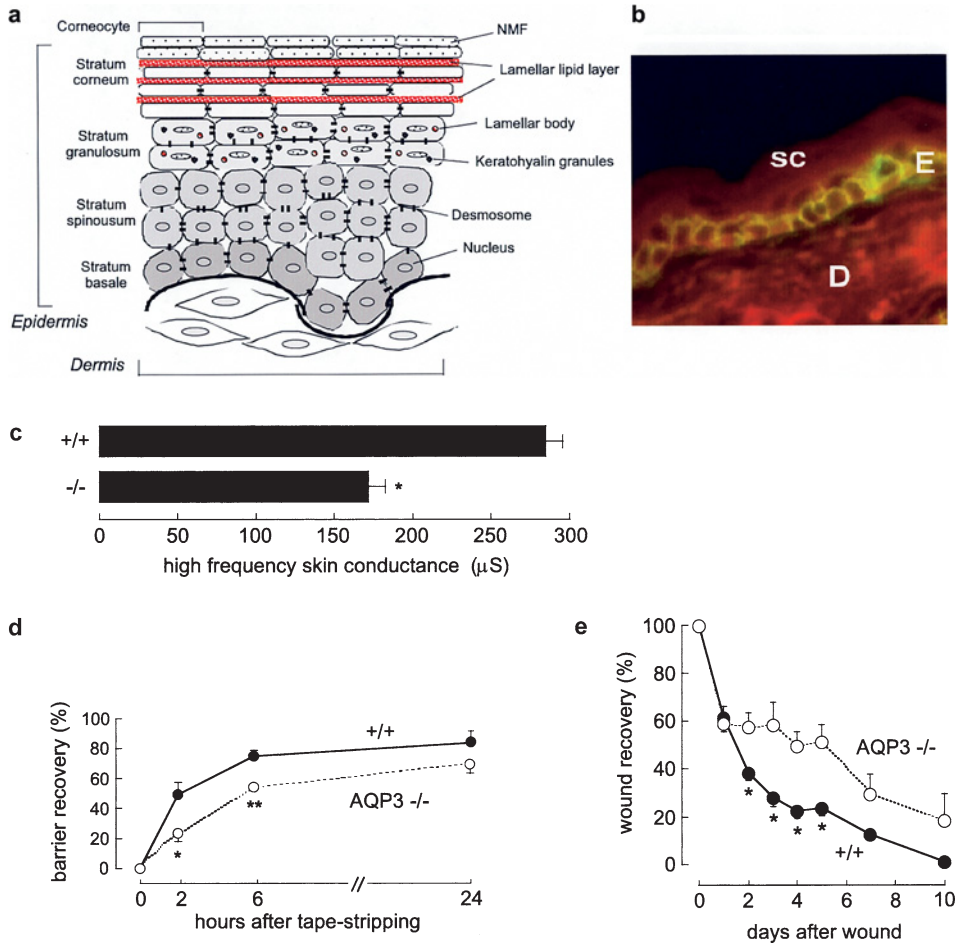

Figure 1. Structure of mammalian epidermis and phenotype in AQP3 deficiency. (*a*) Schematic showing stratum corneum and epidermal layers. (*b*) Immunofluorescence showing AQP3 staining in mouse epidermal cells. E, epidermis; D, dermis; sc, stratum corneum. (*c*) High-frequency superficial skin surface conductance in dorsal skin of hairless wild-type and AQP3 null mice. *, $p < 0.001$. (*d*) Barrier recovery after SC removal by tape-stripping. Transepidermal water loss (TEWL) was measured as an index of barrier function at 0, 2, 6 and 24 h after tape-stripping **, $p < 0.01$; *, $p < 0.05$. (*e*) Wound healing. Two full-thickness punch biopsies (5-mm diameter) were performed on the back, and the area was measured. Data as percentage of initial wound area *, $p < 0.01$. Adapted from [25 and 28].

Further experiments provided evidence about the mechanism by which AQP3 deficiency produces these pleotropic defects in skin function. Fig. 2a shows that AQP3 deletion in mice produced an ~4-fold reduction in osmotic water permeability of epidermal cells, in which cell volume changes were measured in response to osmotic gradients. Glycerol permeability was also reduced in AQP3-deficient keratinocytes as measured by uptake of radiolabeled glycerol (Fig. 2b). A systematic analysis of epidermal and SC morphology (by electron microscopy) and composition (assays of animo acids, lipids, ions, glucose, urea, glycerol etc.) revealed selectively reduced glycerol content in AQP3-deficient SC and epidermis, with normal glycerol concentration in dermis and serum (Fig. 2c). These results suggested reduced glycerol transport from blood into the epidermis in AQP3 deficiency through the basal keratinocytes. Additional observations that provided clues to the mechanism of reduced skin hydration in AQP3 deficiency were that exposure of mice to high humidity or occlusion increased SC hydration in wild-type but not AQP3 null mice, and that SC hydration was similar in both types of mice after exposure to 10% humidity. These findings indicated that water transport through AQP3 is not a rate-limiting factor in transepidermal water loss, so that the water-transporting function of AQP3 was unlikely to account for the skin abnormalities in AQP3 deficiency.

Compelling evidence that the glycerol-transporting function of AQP3 was responsible for the skin abnormalities in AQP3 deficiency came from studies of effects of glycerol 'therapy' on skin phenotype. Glycerol administration, by topical or systemic routes, corrected each of the phenotype abnormalities in AQP3 deficiency, including the reduced SC hydration and delayed barrier recovery [29]. SC glycerol content and water content, as assessed by skin conductance, correlated well for mice placed in a 90% humidity atmosphere and given oral glycerol (Fig. 2d). Together, these data suggested that impaired glycerol transport into the epidermis and SC through the

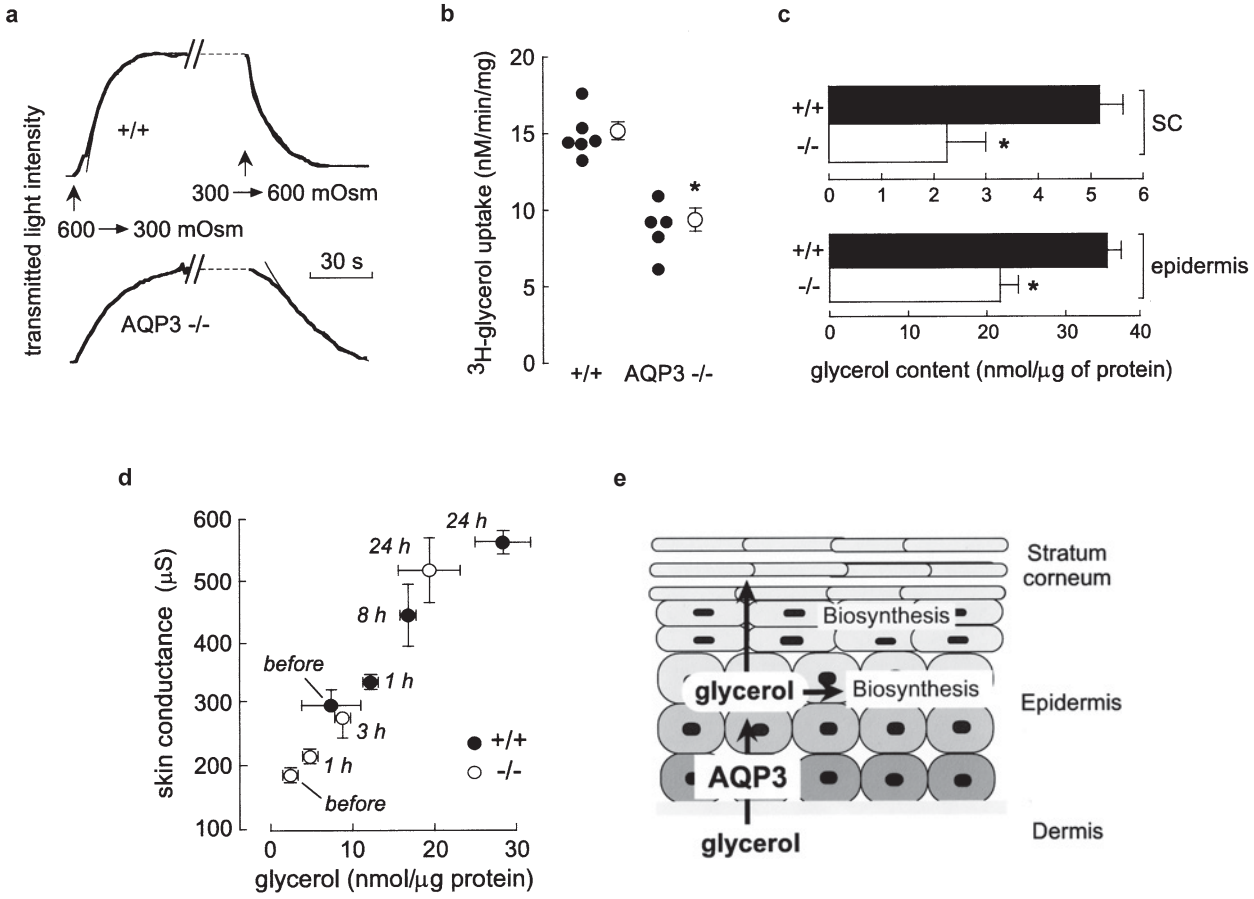

Figure 2. Reduced glycerol permeability and content in AQP3-deficient epidermis. (*a*) Osmotic water permeability of epidermal sheets in which basal keratinocytes were subjected to osmotic gradients. Representative time course of transmitted light intensity (related to epidermal cell volume) in response to changes in perfusate osmolality between 300 and 600 mOsm. Single exponential functions fitted to the data are shown as thin smooth curves. (*b*) [^{3}H]glycerol uptake (at 90 s) measured in freshly isolated suspensions of keratinocytes. *, $p < 0.01$. (*c*) Glycerol content measured in SC and epidermis. (*d*) Correlation between SC glycerol content and skin conductance for wild-type (filled circles) and AQP3 null (open circles) mice in a 90% humidity atmosphere for indicated times. Mice were given glycerol orally ad libitum as their only fluid source (10% for wild-type mice, 2% for AQP3 null mice). (*e*) Proposed mechanism of AQP3 function in epidermis, showing reduced steady-state glycerol content in epidermis and stratum corneum following AQP3 deletion. Adapted from [25, 28 and 29].

relatively glycerol impermeable basal keratinocyte layer results in reduced steady-state epidermal and SC glycerol content (Fig. 2e). Reduced skin glycerol content would then reduce hydration based on the osmolyte properties of glycerol, and impair epidermal biosynthetic functions based on the metabolic/biosynthetic role of glycerol. These findings indicated an important role for AQP3 and glycerol in epidermal function, and provided a rational scientific basis for the long-standing practice of including glycerol in cosmetic and skin medicinal preparations.

Aquaporin-7

AQP7 was cloned independently from human adipose tissue (originally named aquaporin adipose: AQPap) [30] and rat testis [31]. AQP7 is also expressed in sperm [32], kidney [33, 34] and skeletal muscle [35]. Functional analysis in *Xenopus* oocytes demonstrated water, glycerol and urea transport by AQP7 [30, 36]. Another oocyte expression study showed that AQP7 is able to transport arsenite [37].

Role of AQP7 in adipocyte function

AQP7 is expressed abundantly in both white and brown adipose tissue [38]. White adipose tissue is the largest energy storage organ of the body, with >95% of total body lipid in adipose tissue stores as triglyceride. Triglycerides stored in adipocyte are hydrolyzed by hormone-sensitive lipase and adipose triglyceride lipase to glycerol and free fatty acids (FFAs), which are released into the bloodstream and become oxidized throughout the body for energy. Glycerol is an important substrate for hepatic glucose synthesis. Under normal conditions adipocytes have relatively little glycerol kinase activity, though increased glycerol may induce this enzyme [39]. Glycerol-3-phospate (G3P), which is produced from glycerol by the action of glycerol kinase, induces recycling of FFA, resulting in a progressive accumulation of triglycerides in adipocytes. Thus, glycerol is a key metabolite that plays an important role in adipose tissue, largely by serving as the carbon backbone for triglycerides.

AQP7 was inferred to function in adipocytes as a glycerol transporter from the decreased glycerol release in 3T3-L1 adipocytes after AQP7 knock-down by small interfering RNA (siRNA) [38]. The sensitivity of adipose tissue AQP7 expression to fasting/refeeding [38] and insulin deficiency [36, 40], as well as to steroids and adrenergic agonists [41], provided further indirect evidence for involvement of AQP7 in adipocyte metabolism.

Recently, two lines of AQP7 knockout mice were generated with interesting though somewhat different pheno-

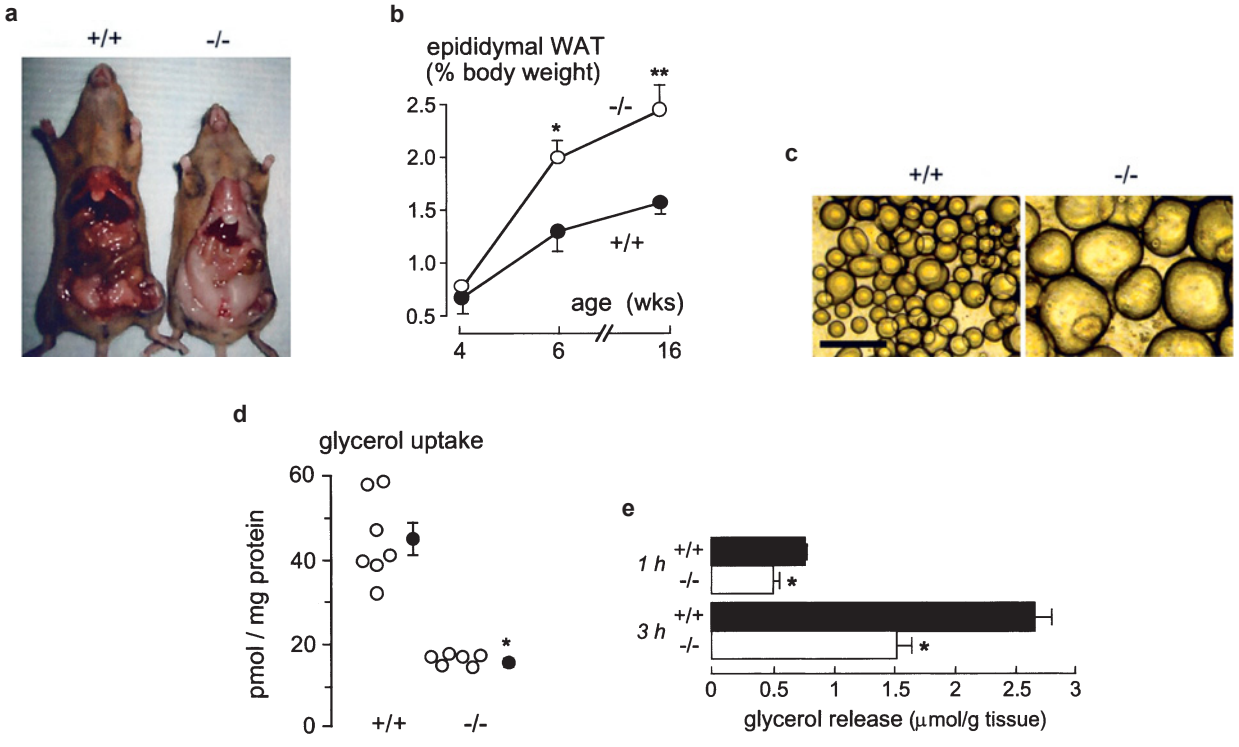

Figure 3. Increased fat mass with adipocyte hypertrophy and reduced glycerol permeability in AQP7-deficient mice. (*a*) Photographs of mice showing increased gonadal fat in AQP7 null mice at age 16 weeks. (*b*) Age-dependent epididymal fat mass (SE, 6 mice). *, $p < 0.05$. **, $p < 0.01$. (*c*) Micrograph of adipocytes isolated from epididymal fat of mice of indicated genotype at age 16 weeks. Bar, 100 μm. (*d*) ^{14}C-glycerol uptake at 5 min in suspensions of adipocytes from mice at age 6 weeks. *, $p < 0.01$. (*e*) Glycerol release from minced adipose tissue. *, $p < 0.01$. Adapted from [43].

types with regard to adipocyte function [42–44]. Studies of AQP7-deficient mice in a CD1 genetic background by our lab showed markedly increased body fat with progressive adipocyte hypertrophy [43]. The body weight of wild-type and AQP7 null mice was similar until age 16 weeks, though fat mass at epididymal, gonadal and mesenteric sites was significantly elevated in AQP7 null mice (Figs. 3a, b). Adipocytes in AQP7 null mice were much larger than those of wild-type mice by 16 weeks (Fig. 3c), and accumulated approximately 3-fold more glycerol and 2-fold more triglycerides. Glycerol permeability in adipocytes from younger mice, which were of comparable size, was significantly reduced by 3-fold in AQP7 deficiency (Fig. 3d), and glycerol release from minced fat tissues was reduced (Fig. 3e). However, lipolysis and lipid synthesis rates were similar in wild-type and AQP7-deficient mice. From these data we suggested that the progressive triglyceride accumulation in AQP7-deficient adipocytes results from reduced plasma membrane glycerol permeability, leading to increased glycerol-3-phosphate and triglyceride biosynthesis with stimulated glycerol kinase [43]. AQP7 null mice in a C57BL/6N genetic background exhibited not only increased fat mass and adipocyte hypertrophy, but also increased body weight and insulin resistance as they aged [44]. Glycerol kinase activity in white adipose tissue from AQP7 null mice was also increased. Together, the findings from the two lines of AQP7 null mice implicate AQP7 as a key element in regulating fat accumulation through glycerol transport in adipocytes. Activation of glycerol kinase by accumulation of substrate glycerol might induce conversion of glycerol to G3P, which could recycle FFAs into triglyceride synthesis in adipocytes (Fig. 4).

A recent study in adipose tissue in lean and obese humans suggested that down regulation of AQP7 in obesity might result in reduced glycerol release from adipocytes [45]. However, a rare human with a genetic defect in AQP7, whose gene lies in the long arm of chromosome 13, did not manifest obesity, but did show reduced elevation of plasma glycerol during exercise [46]. Additional investigations on the phenotype of AQP7 null mice and humans with AQP7 deficiency are needed to better define the role of AQP7 in human obesity and diabetes.

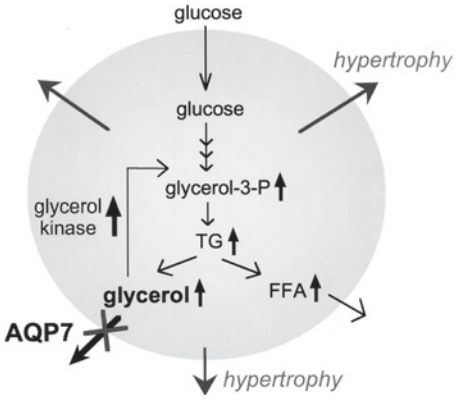

Figure 4. Proposed mechanism for adipocyte hypertrophy in AQP7 deficiency. See text for explanations.

Aquaporin-9

AQP9 is expressed in multiple organs, including liver, testis, brain, leukocytes, epididymis and spleen [47–49]. The original cloning study concluded that AQP9 is permeable to water and many small polar solutes, including carbamides, polyols, purines, pyrimidines and monocarboxylates [47], though a later study showed permeability mainly to urea and glycerol [50]. A study of streptozotocin-induced diabetic mice found AQP9 gene upregulation during fasting and type 1 diabetes mellitus, suggesting the involvement of AQP9 in glucose metabolism and insulin resistance [40]. Another study in rats showed AQP9-facilitated glycerol influx and urea efflux in hepatocytes [51]. However, a recent preliminary report of AQP9 knockout mice revealed normal phenotype [52]. Results of a full analysis, including responses to metabolic stresses, will be needed before it can be concluded that AQP9 is not of physiological significance.

Perspective

The phenotypes of AQP3 and AQP7 null mice suggest interesting new possibilities for clinical therapy. Over the past decade, obesity has gained attention as a public health problem worldwide, with more than half of the adults in the United States being overweight or obese [53]. The involvement of AQP7 in adipocyte metabolism suggests the possibility of new strategies for therapy of obesity, such as pharmacological AQP7 induction to prevent and reverse adipocyte triglyceride accumulation. The involvement of AQP3 in epidermal hydration and biosynthesis suggests the modulation of AQP3 expression or function for therapy of skin diseases associated with excess or reduced epidermal hydration, and perhaps in skin neoplasia associated with excess epidermal cell proliferation.

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