Diet-induced metabolic change induces estrogen-independent allometric mammary growth

Grace E. Berryhill[a], Julia M. Gloviczki[a], Josephine F. Trott[a], Lucila Aimo[a], Jana Kraft[b], Robert D. Cardiff[c], Carly T. Paul[a], Whitney K. Petrie[a], Adam L. Lock[d], and Russell C. Hovey[a,1]

[a]Department of Animal Science, University of California, Davis, CA 95616-8521; [b]Department of Animal Science, University of Vermont, Burlington, VT 05405-0148; [c]Center for Comparative Medicine, University of California, Davis, CA 95616-8521; and [d]Department of Animal Science, Michigan State University, East Lansing, MI 48824-1225

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Lifetime breast cancer risk reflects an unresolved combination of early life factors including diet, body mass index, metabolic syndrome, obesity, and age at first menses. In parallel, the onset of allometric growth by the mammary glands around puberty is widely held to be estrogen (E)-dependent. Here we report that several physiological changes associated with metabolic syndrome in response to a diet supplemented with the *trans*-10, *cis*-12 isomer of conjugated linoleic acid lead to ovary-independent allometric growth of the mammary ducts. The E-independence of this diet-induced growth was highlighted by the fact that it occurred both in male mice and with pharmacological inhibition of either E receptor function or E biosynthesis. Reversal of the metabolic phenotype with the peroxisome proliferator-activated receptor-γ agonist rosiglitazone abrogated diet-induced mammary growth. A role for hyperinsulinemia and increased insulin-like growth factor-I receptor (IGF-IR) expression during mammary growth induced by the *trans*-10, *cis*-12 isomer of conjugated linoleic acid was confirmed by its reversal upon pharmacological inhibition of IGF-IR function. Diet-stimulated ductal growth also increased mammary tumorigenesis in ovariectomized polyomavirus middle T-antigen mice. Our data demonstrate that diet-induced metabolic dysregulation, independently of ovarian function, stimulates allometric growth within the mammary glands via an IGF-IR-dependent mechanism.

Epidemiological data consistently point to an early window of breast development as being sensitive to breast cancer risk factors associated with diet (1), body mass index (BMI) (2), obesity (3), and age at onset of puberty (4). This increased risk may reflect precocious breast development due to an earlier age at puberty (5) as a result of the obesity pandemic (6). However, lines of evidence indicate that this window is before the onset of puberty and first menses and is independent of ovarian function and increased BMI (7). Indeed, several studies point to a period of <10 y of age that confers the greatest risk to radiation-induced breast cancer (8), consistent with the fact that prepubescent female rats are most susceptible to chemical-induced mammary tumorigenesis (9). A role for diet during this period is highlighted by the fact that only girls who were 2–9 y old during the Dutch famine had an increased lifetime risk of developing breast cancer (10). Furthermore, data from a recent prospective study indicate that girls with a low BMI are at a greater risk for developing benign breast disease (11). Combined, lines of evidence such as these point to an early window of breast development before the onset of puberty that could be sensitive to one or more dietary components or diet-induced metabolic change.

The majority of mammary gland (MG) development occurs during postnatal life and primarily initiates with a phase of allometric growth around the onset of puberty that results in extension of the ductal network from the nipple into the surrounding adipose stroma (12). This allometry is widely held to commence in response to the synthesis and secretion of estrogen (E) by the ovaries (13), where ovariectomy ablates ductal elongation (12) whereas E copotentiates the actions of growth hormone (GH) and its stroma-derived paracrine mediator, insulin-like growth factor-I (IGF-I) (14). Much of this regulation is conferred by the surrounding adipose microenvironment of the mammary fat pad, which itself is modified by diet and developmental state (15).

Major clinical signs of metabolic syndrome include impaired insulin signaling associated with visceral adiposity, dyslipidemia, hypertension, and inflammation (16), where hepatic steatosis separately from obesity may more accurately correlate with impaired insulin signaling (17). A diet-dependent model that recapitulates many aspects of the metabolic syndrome involves feeding rodents the *trans*-10, *cis*-12 isomer of conjugated linoleic acid (10,12 CLA), an octadecadienoic fatty acid that is present in foodstuffs either due to the hydrogenation of vegetable oils (18) or at low levels due to biohydrogenation in ruminants (19). When 10,12 CLA is fed to mice, it dysregulates metabolic function coincident with lipoatrophy, transient hypertriglyceridemia, adipose tissue inflammation, hepatic steatosis, and hyperinsulinemia (20–22). The lipoatrophic effect of 10,12 CLA has led to its widespread adoption as a weight-loss supplement (23) that gives rise to elevated plasma triacylglycerol and LDL:HDL cholesterol levels (24). This collection of phenotypes induced by dietary 10,12 CLA therefore provides a useful and defined model of metabolic disruption for studying its effects on MG growth.

Here we report that early allometric growth in the MG of mice occurs independently of estrogenic stimulation following diet-induced metabolic changes resulting from ingestion of 10,12 CLA. These data highlight not only that E-independent allometric growth of the mammary ducts is induced by a dietary component, but also that aspects of the metabolic syndrome elicit this growth, which may increase E-independent breast cancer risk.

Results

Dietary 10,12 CLA Increases Mammary Ductal Growth. Morphology of the MG from ovary-intact female Balb/cJ mice fed a diet containing 1% 10,12 CLA after weaning at 21 d was first analyzed at 35 d. Mice fed 10,12 CLA for 14 d had greater ductal elongation compared with mice fed the control diet ($P < 0.05$; Fig. 1). The MG of mice fed 10,12 CLA accumulated 10,12 CLA ($P < 0.05$; Table S1) coincident with reduced adiposity measured as wet MG mass ($P < 0.05$; Table S2). We also investigated whether dietary 10,12 CLA altered ductal elongation in ovariectomized (OVX) mice supplemented with E for 14 d. Similar to ovary-intact mice, OVX mice treated with E tended to have increased ductal growth in response to 10,12 CLA ($P = 0.09$; Fig. S1).

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[1]To whom correspondence should be addressed. E-mail: rchovey@ucdavis.edu.

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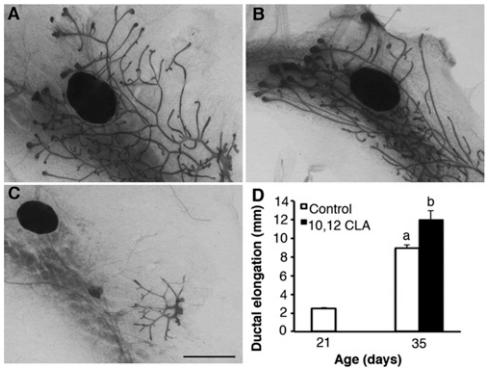

Fig. 1. Dietary 10,12 CLA stimulates ductal elongation in the mammary glands of ovary-intact peripubertal mice. Representative mammary gland whole mounts of female Balb/cJ mice weaned onto either the (*A*) control or the (*B*) 10, 12 CLA diet at 21 d and then euthanized at 35 d. *C* is a representative whole mount at 21 d. (*D*) Ductal elongation was measured as the farthest distance that the duct termini extended from the nipple. (Scale bar, 2 mm.) Data are means ± SEM (n = 4–5/group). [a,b]Means with different superscripts are different ($P < 0.05$).

Dietary 10,12 CLA Induces Ovary- and E-Independent Mammary Ductal Growth. Surprisingly, we found that mice ovariectomized at 21 d and then fed 10,12 CLA for another 21 d had significant elongation of the ductal network ($P < 0.05$; Fig. 2) that extended to the supramammary lymph node, comparable to that typical in ovary-intact females around 33 d of age (25). The ductal morphology was characterized by enlarged, bifurcated, and proliferative terminal end buds (TEB) (Fig. 2*E*) that contrasted to the blunted, quiescent ductal termini in control-fed females (Fig. 2*A*). The 10,12 CLA-induced TEB development was evident just 7 d after commencement of the diet (Fig. 2*A*). Ductal elongation in response to 10,12 CLA in OVX mice (Fig. 2*B*) and the overall ductal area (Fig. 2*C*) increased over time to reach a maximum by 35 d ($P < 0.05$; Fig. 2), which was sustained at 42 and 63 d. The MG of mice fed 10,12 CLA also had a lower mass and adiposity after they ingested 10,12 CLA for only 7 d and increased liver mass by 35 d of age ($P < 0.05$; Fig. S2). Given that allometric growth in the MG was originally defined as "a growth coefficient relative to metabolic size" (13), we used this approach to establish whether 10,12 CLA-stimulated ductal growth in OVX females was allometric. Indeed, OVX mice fed 10,12 CLA had a growth coefficient of 3.95 compared with 0.60 for control-fed mice (Fig. 2*D*). These values are similar to those reported for allometric growth in ovary-intact mice and for isometric growth in OVX mice, respectively (13).

We also determined whether this effect of 10,12 CLA was strain-specific by feeding 10,12 CLA to 129SVE, C57BL/6, and FVB mice for 21 d post ovariectomy. The 10,12 CLA-induced MG phenotype differed subtly between strains. Ductal elongation increased approximately twofold in Balb/cJ mice ($P < 0.05$; Fig. S3*I*) whereas ductal area nearly doubled in 129SVE mice ($P < 0.05$; Fig. S3*J*). Similarly, epithelial area increased in 10,12 CLA-fed FVB mice ($P < 0.05$; Fig. S3*M*). There was no change in ductal elongation or area in C57BL/6 mice fed 10,12 CLA. Mass of the MG was reduced by 10,12 CLA in all strains whereas liver mass increased consistently ($P < 0.05$; Fig. S3 *K* and *L*).

Given the ovary independence of this growth stimulation, we also assessed whether dietary 10,12 CLA stimulated ductal elongation in the MG of peripubertal male 129SVE mice. Males of this strain, unlike Balb/cJ, maintain a ductal rudiment about the nipple. Indeed, ductal area was increased after 21 d of dietary 10,12 CLA versus the control diet ($P < 0.05$; Fig. 3). The amount of outgrowth induced by 10,12 CLA was similar to that recorded in OVX females.

To further confirm the E independence of this diet-induced phenotype, we either blocked estrogen receptor (ER) activity in 10,12 CLA-fed OVX mice by coadministering ICI 182,780 or inhibited aromatization by coadministering letrozole. Neither compound affected 10,12 CLA-induced ductal elongation ($P < 0.05$; Fig. 4). The efficacy of ICI 182,780 was confirmed by its ability to suppress E-induced uterotrophy ($P < 0.05$; Fig. S4). Any potential for adrenal-stimulated growth was excluded when similar responses to 10,12 CLA were recorded in adrenalectomized, OVX female mice ($P < 0.05$; Fig. 5), despite lower corticosterone levels ($P < 0.05$; Fig. S4). Combined, our findings clearly establish that dietary 10,12 CLA induces E-independent allometric mammary growth.

Diet Induces Mammary Growth via Metabolic Dysregulation and the Insulin/IGF-I Axis. We considered which aspects of diet-induced metabolic dysregulation could induce allometric mammary growth where insulin-responsive IGF-I signaling regulates ductal growth (14). Intake of 10,12 CLA increased serum insulin in OVX female Balb/cJ and male 129SVE mice by 42 or 43 d of age, respectively ($P < 0.05$; Fig. 6 *A* and *B*). By contrast, 10,12 CLA fed

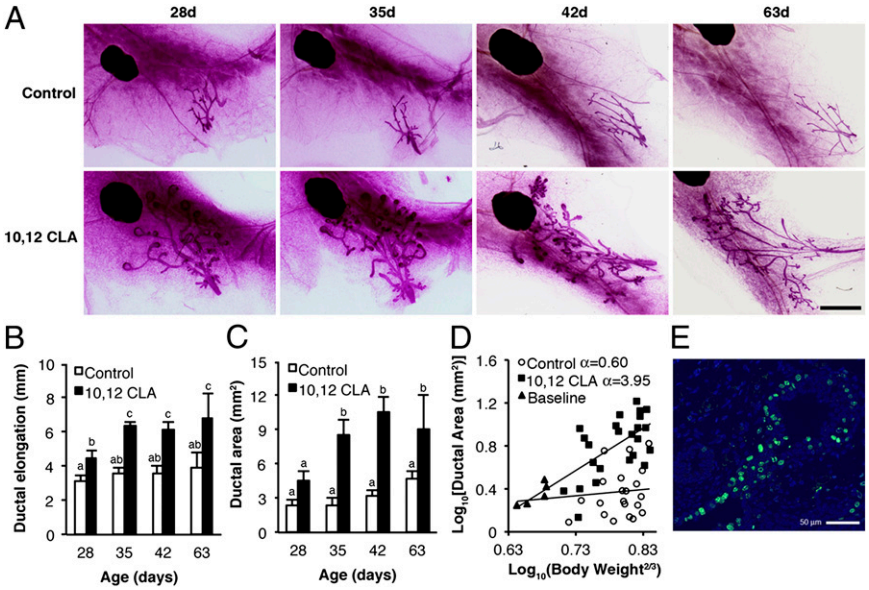

Fig. 2. Dietary 10,12 CLA stimulates mammary gland growth in OVX peripubertal mice. (*A*) Representative mammary gland whole mounts from OVX Balb/cJ mice fed either the control or the 10, 12 CLA diet from 22 d and euthanized at 28, 35, 42, or 63 d. (Scale bar, 2 mm.) (*B*) Ductal elongation measured per Fig. 1. (*C*) Ductal area measured as the polygon area surrounding the ductal network. (*D*) Linear regression of $\log_{10}$ (Ductal area) and $\log_{10}$ (Body weight$^{2/3}$) from OVX mice fed either the control or the 10,12 CLA diet from 22 d until euthanized at 28, 35, or 42 d of age. A subset of mice was euthanized at 21 d (Baseline). Ninety-five percent confidence limits for control and 10,12 CLA groups were −0.85–2.04 and 2.62–5.28, respectively. (*E*) Representative terminal end bud from a 42-d-old OVX mouse fed 10,12 CLA for 21 d. Proliferating cells were detected by 5-ethynyl-2′-deoxyuridine histochemistry (green), overlaid with DAPI (blue). Data are means ± SEM (n = 4–10/group). [a,b,c]Means with different superscripts are different ($P < 0.05$).

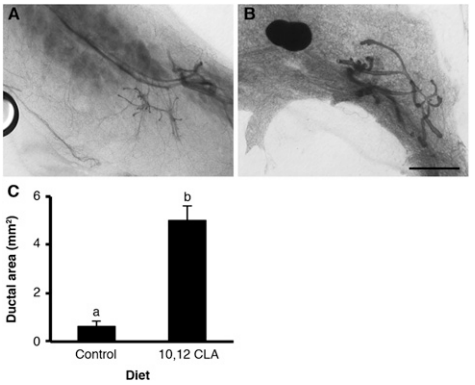

Fig. 3. Dietary 10,12 CLA stimulates the mammary glands of peripubertal male mice. Representative mammary gland whole mounts from 129SVE male mice weaned onto either the (*A*) control or the (*B*) 10,12 CLA diet at 21 d and euthanized at 43 d. (Scale bar, 2 mm.) (*C*) Ductal area determined per Fig. 2. Data are means ± SEM (n = 7/group). [a,b]Means with different superscripts are different ($P < 0.05$).

to OVX mice did not alter serum IGF-I levels that independently declined with age ($P < 0.05$; Fig. 6*C*). Interestingly, serum IGF-I levels in male 129SVE mice were lower after the 10,12 CLA diet ($P < 0.05$; Fig. 6*D*). Given key roles for local IGF axis components in the MG (14), we also examined their expression following dietary 10,12 CLA. Although there was no main effect of diet on IGF-I mRNA levels in the MG ($P = 0.08$), 10,12 CLA suppressed its expression at 28 d ($P < 0.05$; Fig. 7*A*). The level of insulin-like growth factor-I receptor (IGF-IR) mRNA in the MG of 10,12 CLA-fed females was elevated at 42 d, but not at 28 or 35 d of age ($P < 0.05$; Fig. 7*B*). There was a significant negative main effect of 10,12 CLA on insulin receptor (IR)-B mRNA levels ($P < 0.05$; Fig. 7*C*), whereas there was no main effect of

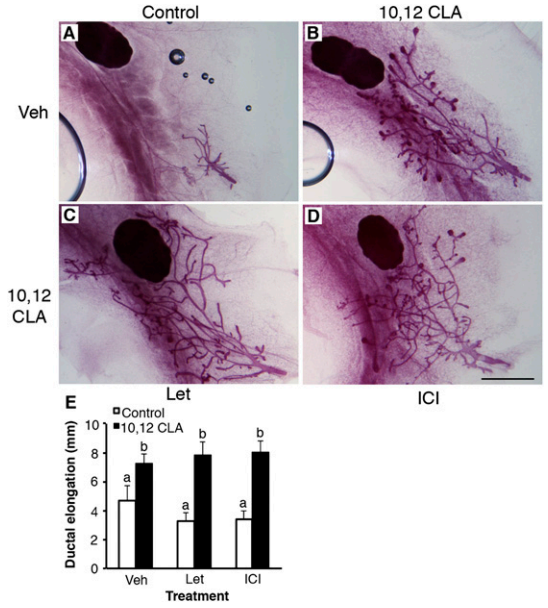

Fig. 4. Ovary-independent mammary growth induced by dietary 10,12 CLA is not mediated by the E receptor or endogenous E biosynthesis. Representative mammary gland whole mounts from ovariectomized Balb/cJ mice fed either the (*A*) control or (*B*) 10,12 CLA diet from 22 d and euthanized at 42 d. Mice were coadministered daily injections of (*A* and *B*) sesame oil vehicle (Veh), (*C*) letrozole (Let), or (*D*) ICI 182,780 (ICI). (Scale bar is 2 mm.) (*E*) Ductal elongation measured per Fig. 1. Data are means ± SEM (n = 4–6/group). [a,b]Means with different superscripts are different ($P < 0.05$).

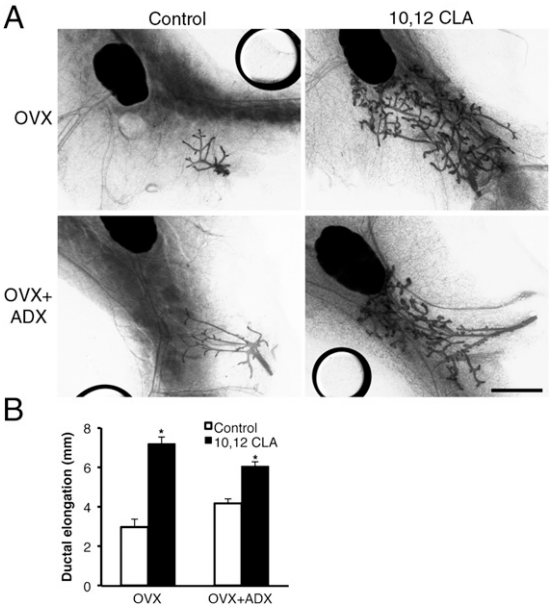

Fig. 5. Dietary 10,12 CLA stimulates mammary growth in peripubertal mice independently of both the ovaries and the adrenal glands. (*A*) Representative mammary gland whole mounts from Balb/cJ mice either ovariectomized (OVX) or ovariectomized and adrenalectomized (OVX+ADX), at 21 d. Mice were then fed either the control or the 10,12 CLA diet for a further 21 d. (Scale bar, 2 mm.) (*B*) Ductal elongation measured per Fig. 1. Data are means ± SEM (n = 3–5/group). *Within a surgical treatment group control-fed and 10,12 CLA-fed mice are different ($P < 0.05$).

diet on IR-A mRNA levels, although they decreased with age ($P < 0.05$; Fig. 7*D*). There was a trend toward a suppressive main effect of 10,12 CLA on IGF-II mRNA levels (Fig. 7*E*; $P = 0.06$).

Functionality of the insulin/IGF axis also occurs at the post-transcriptional level (26). Western blot analysis revealed that IGF-IR protein abundance was increased in the MG of 35- and 42-d-old OVX mice fed 10,12 CLA ($P < 0.05$; Fig. 7*F* and Fig. S5*A*). This increase was manifest in the epithelial compartment, given that IGF-IR abundance was not altered in the contralateral epithelium-free mammary fat pad ($P > 0.05$; Fig. S5*B*). Concordant with the mRNA expression results, both IRα and IRβ protein levels were reduced in the intact MG of 10,12 CLA-fed mice at 42 d of age ($P < 0.05$; Fig. 7 and Fig. S5 *C–F*).

Insulin Sensitization and IGF-IR Blockade Reverses Diet-Induced Mammary Growth. Given that many aspects of the metabolic syndrome can be reversed by therapeutic sensitization to insulin, we coadministered the peroxisome proliferator-activated receptor-γ (PPARγ) agonist rosiglitazone (Rosi) to OVX mice fed the 10,12 CLA diet until 42 d of age. Ductal growth induced by 10,12 CLA was abolished by Rosi ($P < 0.05$; Fig. 8 *A–E*). In parallel, Rosi ameliorated the effect of 10,12 CLA on MG mass ($P < 0.05$; Fig. S6), negated the 10,12 CLA-stimulated hyperinsulinemia ($P < 0.05$; Fig. 8*F*), and abrogated the elevated IGF-IR protein levels in the MG ($P < 0.05$; Fig. 7*F* and Fig. S5*A*). Given these data and the aforementioned changes in components of the insulin/IGF-I axis including increased IGF-IR abundance, we next coadministered the IGF-IR inhibitor picropodophyllotoxin (PPP) to OVX mice fed the control or 10,12 CLA diet. Treatment with PPP completely blocked 10,12 CLA-induced ductal elongation ($P < 0.05$; Fig. 9), which was similar to the effect of Rosi. These data indicate that E-independent growth stimulation occurs alongside altered IGF-IR function and dysregulated insulin action.

Diet-Induced Mammary Growth Facilitates the Growth of Transformed Epithelium. We sought to establish whether 10,12 CLA-induced growth of the normal MG would also stimulate growth of

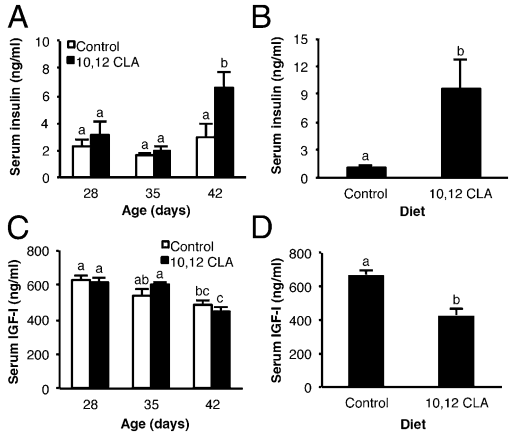

Fig. 6. Dietary 10,12 CLA elevates serum insulin in OVX peripubertal mice. Serum insulin (*A* and *B*) and IGF-I (*C* and *D*) were measured by ELISA. (*A* and *C*) OVX Balb/cJ mice were fed either the control or the 10,12 CLA diet from 22 d to 28, 35, or 42 d. (*B* and *D*) Male 129SVE mice were fed either the control or the 10,12 CLA diet from 21 to 43 d. Data are means ± SEM (n = 4–5/group). [a,b,c]Means with different superscripts are different ($P < 0.05$).

genetically transformed mammary epithelium in polyomavirus middle T-antigen (PyMT) transgenic mice that are predisposed to early onset mammary tumors during allometric growth (27). Heterozygous PyMT mice were ovariectomized at 21 d and fed the control or 10,12 CLA diets for 21 d. Whole-mount analysis highlighted that the 10,12 CLA diet markedly increased the area of epithelial hyperplasia ($P < 0.05$; Fig. 10) to an extent that was proportional to that recorded in the MG of OVX females (Fig. 2). There was no difference in the histopathology of these hyperplasias.

Discussion

Dogma asserts that the mammary ducts undergo early allometric growth around the onset of puberty in response to an increase in ovarian E synthesis (12, 14), consistent with the widespread demonstration that ovariectomy abolishes mammary growth. Decades of research have established that ductal growth reflects the concerted actions of E, progesterone (P), and GH (12). Specifically, GH stimulates the local synthesis of IGF-I by the mammary stroma (28) whereas E enhances this effect of GH (29) by activating GH-inducible stromal ER (30). In turn, IGF-I stimulates TEB formation (14) that is enhanced by E (29). Maximal growth of the mammary ducts in response to IGF-I ultimately depends on facilitation by either E (29) or P, while the combination of E, P, and IGF-I is essential for alveolar development (31).

Here we reveal that allometric ductal growth in OVX peripubertal mice initiates in response to a dietary component, namely 10,12 CLA. Most notably, this growth is independent of ovarian stimulation, any action of E via its receptor, or systemic E biosynthesis. Identification of this E-independent mechanism is a major extension of studies showing that ovary-intact mice fed 10,12 CLA had increased TEB number (32) or premature lobuloalveolar development (33).

Epidemiological studies have consistently highlighted an as-yet-unclear relationship between early life diet (34, 35), obesity (3), BMI (2), and breast cancer risk. Dietary fat intake has generally failed to account for breast cancer risk across numerous epidemiological analyses (36), although consumption of hydrogenated fats was recently associated with a greater risk (37). We found that dietary 10,12 CLA induced allometric growth coincident with aspects of metabolic dysregulation including lipoatrophy, hyperinsulinemia, and hepatic steatosis, whereas others have also described adipose inflammation (22) in response to this diet. We posit that dietary 10,12 CLA confers its effects on the MG by inducing aspects of the metabolic syndrome that have been repeatedly linked to increased breast cancer risk (38) concurrent with the obesity epidemic, particularly via its effects in young girls (6). Our finding that the MG of various strains of mice differed in their response to 10,12 CLA despite having similar metabolic changes supports a potential genetic basis for how diet and/or diet-induced metabolic dysregulation affects breast cancer risk in different populations (39).

A candidate role for the insulin/IGF-I axis in facilitating this diet-induced phenotype was manifest at several levels. We suggest that hyperinsulinemia in response to dietary 10,12 CLA may have contributed to increased epithelial growth, given that systemic and local IGF-I levels were unaffected. Such a mechanism would coincide with the ability of hyperinsulinemia to stimulate growth of the mammary ducts and mammary tumors in a nonobese model of type 2 diabetes (40). Furthermore, postmenopausal women with elevated insulin levels are at a higher risk for developing breast cancer independently of any effect of circulating E (41) whereas breast cancer patients with type 2 diabetes have a poorer outcome (42). Our finding that the insulin-sensitizing actions of Rosi completely reversed the effects of 10,12 CLA on mammary growth lends support to the recent proposal that metformin may confer benefits as an adjuvant breast cancer therapy (38). Despite these lines of evidence, any potential for insulin to promote normal mammary development remains unclear given that IGF-I–deficient mice fail to undergo mammary growth (14).

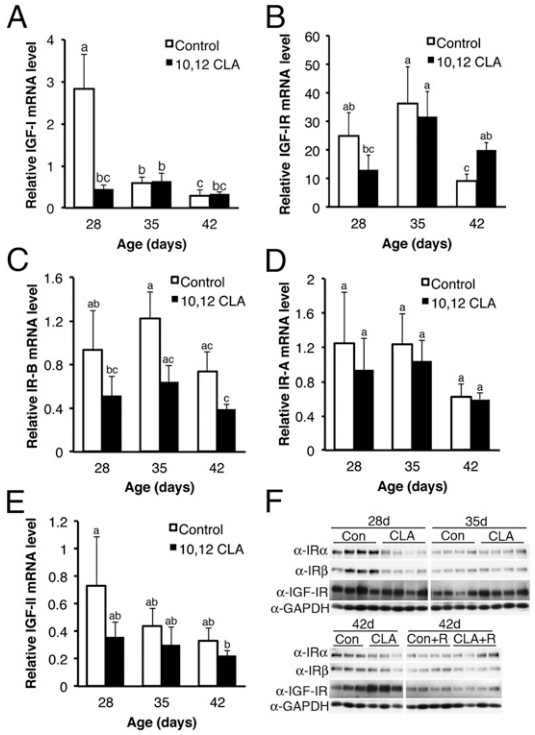

Fig. 7. Dietary 10,12 CLA alters gene and protein expression profiles for components of the insulin/IGF axis in mammary glands of OVX mice. Total RNA from the mammary glands of OVX Balb/cJ mice fed either the control or the 10,12 CLA diet from 22 d to 28, 35, or 42 d was analyzed for mRNA expression of (*A*) IGF-I, (*B*) IGF-IR, (*C*) IR-B, (*D*) IR-A, and (*E*) IGF-II. Data are means ± SEM (n = 3–7/group). [a,b,c]Means with different superscripts are different ($P < 0.05$). (*F*) Western blot analysis of total proteins from the mammary glands of OVX Balb/cJ mice fed either the control or the 10,12 CLA diet from 22 d to 28, 35, or 42 d for IRα, IRβ, IGF-IR, and GAPDH. A subset of OVX mice was administered rosiglitazone (R) concurrent with the diets.

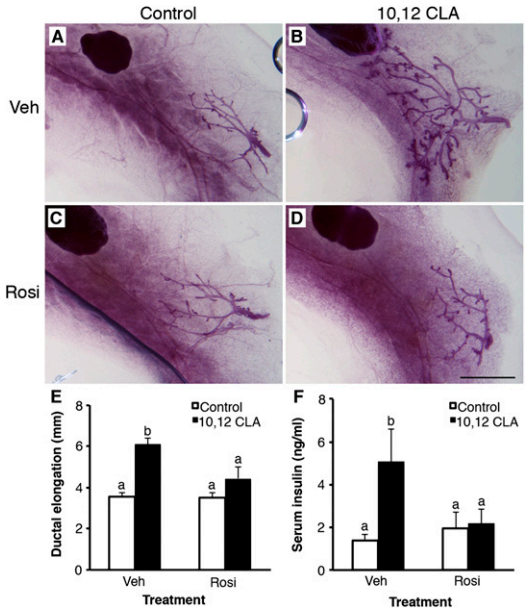

Fig. 8. PPARγ agonist rosiglitazone inhibits ovary-independent 10,12 CLA-induced mammary gland growth. (*A*–*D*) Representative mammary whole mounts from ovariectomized Balb/cJ mice fed either the control (A, C) or the 10,12 CLA diet (*B* and *D*) from 22 d and coadministered either DMSO (Veh; *A* and *B*) or rosiglitazone (Rosi; *C* and *D*) for a further 21 d. (Scale bar, 2 mm.) (*E*) Ductal elongation measured per Fig. 1. (*F*) Serum insulin concentrations. Data are means ± SEM (n = 3–7/group). [a,b]Means with different superscripts are different ($P < 0.05$).

Our data are also consistent with a potential role for cross-talk between insulin and IGF-I receptors during MG development (43). Interestingly, levels of the IGF-IR protein, more so than expression of its mRNA, were increased in the MG of 10,12 CLA-fed mice. A similar situation was recorded during pancreatic neuroendocrine carcinogenesis whereby levels of IR and IGF-IR mRNA were increased only slightly versus a pronounced induction at the translational level (26). Furthermore, functionality of the IGF-IR is required for the diet-induced mammary growth recorded here as highlighted by the fact that it was reversed by PPP, consistent with the essential role of the IGF-IR in mediating normal mammary ductal development (44). These data align with recent findings from a kinome scan pointing to an insulin/IGF-I receptor-dependent pathway for E-independent growth of breast cancer cells (45), reinforcing key roles for the IGF-IR in breast cancer and its therapeutic targeting that likely converges with its many candidate roles during the metabolic syndrome (46, 47). Whether certain dietary constituents such as 10,12 CLA or metabolic dysregulation impinge on mammary growth during puberty via the synergistic relationships that exist between IGF-I and E (29) or P (31) remains to be established.

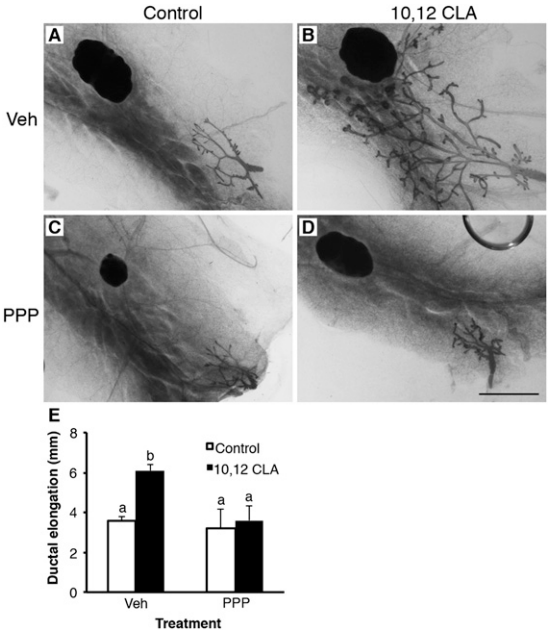

Fig. 9. Blockade of IGF-IR abrogates ovary-independent 10,12 CLA-induced mammary growth. (*A*–*D*) Representative mammary whole mounts from ovariectomized Balb/cJ mice fed either the control (A, C) or the 10,12 CLA diet (*B* and *D*) from 22 d and coadministered either DMSO (Veh; *A* and *B*) or PPP (*C* and *D*) for a further 21 d. (Scale bar, 2 mm.) (*E*) Ductal elongation measured per Fig. 1. Data are means ± SEM (n = 3–6/group). [a,b]Means with different superscripts are different ($P < 0.05$).

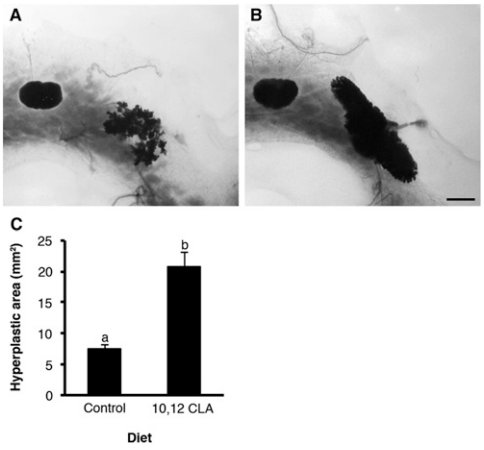

Fig. 10. Dietary 10,12 CLA accelerates tumorigenesis in transgenic mice expressing the PyMT antigen. (*A* and *B*) Representative mammary whole mounts from ovariectomized mice heterozygous for PyMT fed either the (*A*) control or (*B*) 10,12 CLA diet from 22 to 42 d. (Scale bar, 2 mm.) (*C*) Average total hyperplastic area. Data are means ± SEM (n = 5/group). [a,b]Means with different superscripts are different ($P < 0.05$).

In conclusion, our findings highlight a striking link between diet, metabolic dysregulation, and allometric MG growth that is independent of estrogenic stimulation. These results lend support to increasing evidence suggesting a relationship between breast cancer risk and early life events that clearly include dietary components and their effects on aspects of metabolic dysregulation.

Materials and Methods

All details regarding mice, strains, diets, surgery, treatments, and housing conditions are outlined in *SI Materials and Methods*. Diets were isocaloric and based on a modified AIN93G diet that contained 15% fat by weight, with 10,12 CLA replacing 1% fat by weight. The 10,12 CLA content in the experimental diet was 6.81% of total fatty acids, whereas it was undetectable in the control diet (Table S3). Mice were fed the control diet for 1 d following ovariectomy and then randomly assigned to either the control diet or 10,12 CLA. Daily injections started concurrently with diet assignments. All details of the analysis of mammary gland development, circulating hormones, fatty acids, gene expression (Table S4), proteins, and statistics are outlined in *SI Materials and Methods*.

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