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Atypical xanthomatosis in apolipoprotein E-deficient mice after cholesterol feeding

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Abstract

Apolipoprotein (apo) E-deficient mice were fed a hypercholesterolemic diet for 14 weeks. Mean serum cholesterol levels rose to 37.5 mM. Upon complete necropsy, massive xanthomatous lesions were noticed in various tissues, with a predilection for subcutaneous and peritendinous tissues, while control animals on the same diet (3.4 mM serum cholesterol) and apo E-deficient mice on a regular chow diet (20 mM serum cholesterol) did not show such lesions. Also, apo E3-Leiden transgenic mice fed a high fat diet, with 60 mM of serum cholesterol, did not exhibit any xanthomatosis. The xanthomatous lesions found in the *ApoE* knock-out mouse clearly differed in location from xanthomas previously found in low density lipoprotein receptor-deficient mice. We conclude that the lack of apo E results in atypical disseminated xanthomatosis, suggesting that apo E has an important role in determining the tissue distribution of cholesterol deposition.

Keywords: Lipoprotein metabolism; Cholesterol deposition; Familial dysbetalipoproteinemia; Hypercholesterolemia; Mouse model; Gene targeting

1. Introduction

Familial dysbetalipoproteinemia (FD), or type III hyperlipoproteinemia, is a genetic disorder of lipoprotein metabolism predisposing to premature atherosclerosis. FD is defined by high levels of plasma cholesterol and triglyceride, due to the ac-

cumulation of chylomicron and very low density lipoprotein (VLDL) remnants [1]. The primary metabolic defect of FD is mostly the presence of mutant forms of apolipoprotein (apo) E, resulting in a disturbed receptor-mediated clearance of lipoprotein remnants by the liver [2]. In addition, FD may also occur as a consequence of a complete lack of apo E [3–6].

Recently, two types of mouse models for FD have been generated [7–11]. One model comprises

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conventional transgenic mice, overexpressing a mutant form of human apo E, which in man causes a dominantly inherited form of the disease [7,8]. Both the apo E3-Leiden and the apo E (Cys 112→Arg; Arg 142→Cys) transgenic mice display increased levels of cholesterol and triglyceride in the serum VLDL and low density lipoprotein (LDL) fractions. The other model for FD represents knock-out mice with a true null mutation in the endogenous mouse *ApoE* gene [9–11]. These apo E-deficient mice show a much more severe phenotype than the transgenics, with high plasma cholesterol levels, even when fed a chow diet. Diet-induced hypercholesterolemia and atherosclerosis have been described for both FD models [12–14].

The occurrence of xanthomas, an accumulation of fat-laden histiocytes in various tissues, forms a major clinical feature of hyperlipoproteinemia in general. More specifically, about 50% of patients with FD display tuberous xanthomas on the elbows and/or yellowish lipid deposits in the creases of the palms of the hands, the so-called xanthomata striata palmaris. Both kinds of xanthomas are pathognomonic for FD [15]. In addition, tendinous lesions are sometimes observed in FD patients.

Familial hypercholesterolemia (FH), which is characterized by an elevated plasma LDL-cholesterol level, due to a defective LDL receptor (LDLR), is often accompanied by typical tendinous xanthomas in the achilles tendon and in the extensor tendons of the hands. Recently, Ishibashi et al. showed similar grossly observable xanthoma formation in cholesterol-fed LDLR-negative mice, demonstrating that mice are also able to develop xanthomas [16]. In contrast, the appearance of xanthomas has not been described so far in mice lacking apo E.

Here we report that, on histologic examination, apo E-deficient mice fed a hypercholesterolemic diet for 14 weeks do develop massive xanthomatosis in all kinds of tissues, with a predilection for subcutaneous and peritendinous tissue. On the macroscopic level, xanthomas could only be observed after a much longer (6.5 months) dietary treatment. We hypothesize that the lack of apo E results in early atypical cholesterol deposition, sug-

gesting that apo E serves an important role in determining the tissue distribution of xanthomatosis.

2. Methods

2.1. Mice

Apo E-deficient mice were created by homologous recombination in embryonic stem cells as described [9] and were hybrids between the C57BL/6 and 129 Sv strains. C57BL/6 mice were used as controls. Apo E3-Leiden transgenic mice from the high expressor line #181 had previously been generated [7]. Experiments were performed with males only. Mice were bred and housed under standard conditions in the transgenic animal facilities of the Medical Faculty in Nijmegen (*ApoE* knockouts) and Leiden (controls and apo E3-Leiden transgenic mice).

2.2. Diet

Mice were allowed access to food and water ad libitum. The following diets were used. (a) A regular breeding chow diet (RMH-B) containing 6.2% fat. (b) A mild high fat/cholesterol (HFC) diet containing 15% cocoa butter, 0.25% cholesterol, 40.5% sucrose, 10% cornstarch, 1% corn oil, and 6% cellulose. (c) A severe high fat/cholesterol diet (HFC0.5%) containing 15% cocoa butter, 1% cholesterol, 0.5% cholate, 40.5% sucrose, 10% cornstarch, 1% corn oil, and 4.7% cellulose (all percentages are by weight). The semi-synthetic diets HFC and HFC0.5% were composed essentially according to [17]. All diets were purchased from Hope Farms, Woerden, The Netherlands.

2.3. Histological analysis

Mice were sacrificed at 6–7 months of age, after 14 weeks of diet feeding. Complete necropsy including microscopic examination was performed. Tissues were fixed in 10% neutral-buffered formalin, processed and embedded in paraffin. Three-micrometre sections were routinely stained with hematoxylin–phloxine–safran (HPS).

3. Results

Apo E-deficient mice and controls were fed a mild hypercholesterolemic diet (HFC) for 14 weeks.

Thereafter, four null mutants and two controls were sacrificed and a complete necropsy was performed. Mean serum cholesterol levels were 37.5 mM and 3.4 mM, respectively. Massive xanthomatosis was observed in various tissues in all four apo E-deficient mice after cholesterol feeding as described below, while no xanthomas could be observed in controls on the same diet (Fig. 1).

Extensive xanthomatous lesions were observed in the skin (compare Fig. 1A for controls with Fig. 1B for apo E-deficient mice; arrowheads indicate early lesions) and subcutaneous tissues, extending into the salivary glands (Fig. 1C). Lesions were also seen in thyroid glands, muscles, periosteum and upper mediastinum, including thymus (not shown). Fig. 1D shows a higher magnification of a xanthomatous lesion of the skin, with cholesterol clefts (arrowheads), multinucleated giant cells (short arrow) and necrosis (long arrow). Furthermore, xanthomatous lesions were found in periarticular and peritendinous tissues (Fig. 1E, arrows) as well as in the submucosa of esophagus and forestomach (see Figs. 1F and 1G for normal and mutant forestomach, respectively; note the broadened submucosa in the null mutant). Remarkably, the lesions abruptly stopped at the transition from squamous epithelium to glandular epithelium (Fig. 1H).

Locations with mild xanthomatous lesions were found in the mesothelium of the bladder and kidney, and in the nasal mucosa (not shown). In the brain, lesions were present at the origin of the choroid plexus (Fig. 1I, arrowhead), subependymal in the roof of the third ventricle (Fig. 1I, arrow) and in the meninges (Fig. 1J). The meninges of the spinal cord were also affected. In the sinuses of lymph nodes, many giant cells and cholesterol clefts were observed (results not shown).

Similarly, two age-matched apo E-deficient mice on chow (serum cholesterol level of 20 mM) and three high expressor apo E3-Leiden transgenic mice, fed a severe hypercholesterolemic diet (HFC 0.5%) for 14 weeks (serum cholesterol level of 60 mM) [12], were studied. Neither microscopic nor macroscopic analysis could reveal any xanthomas in these animals.

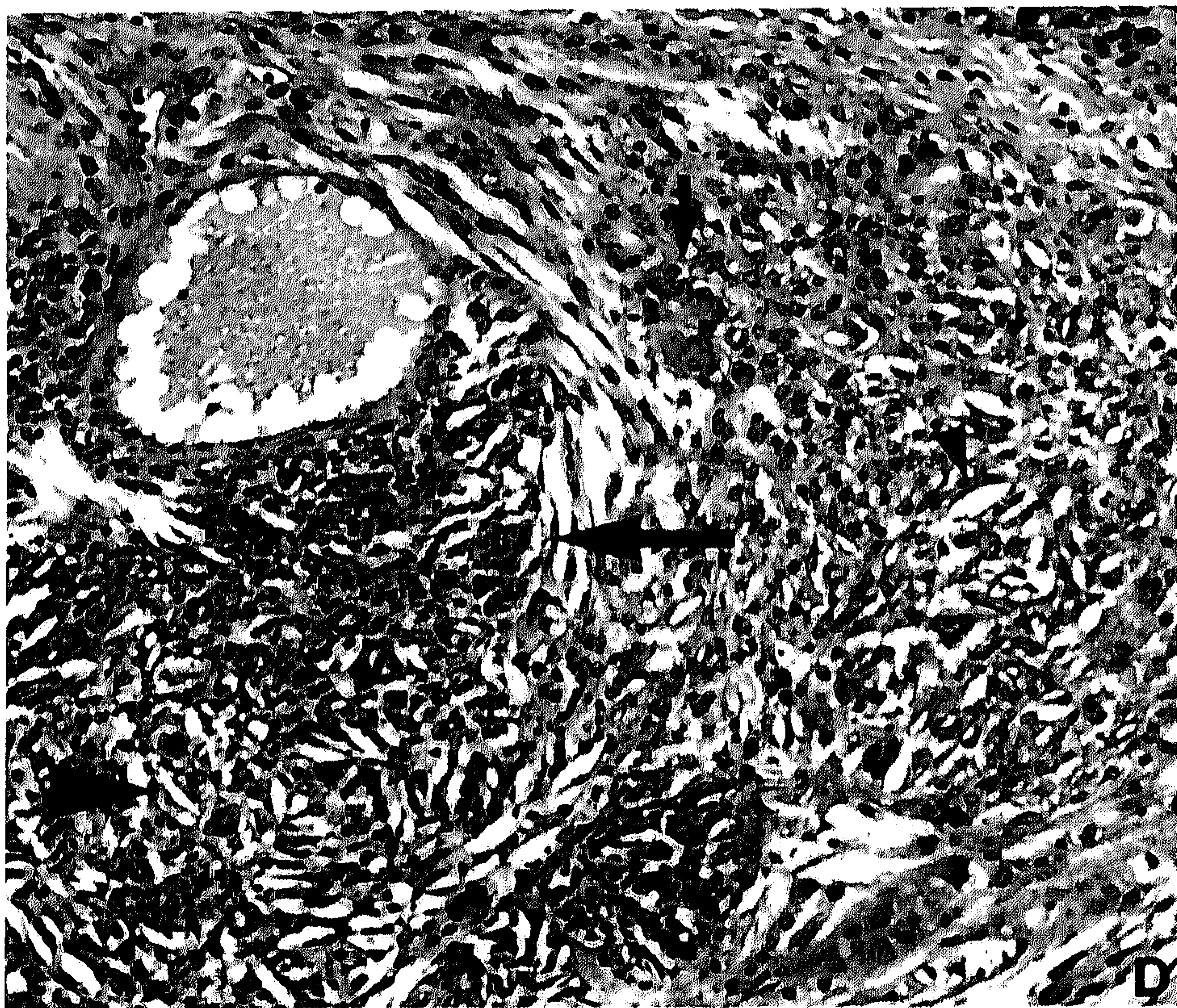
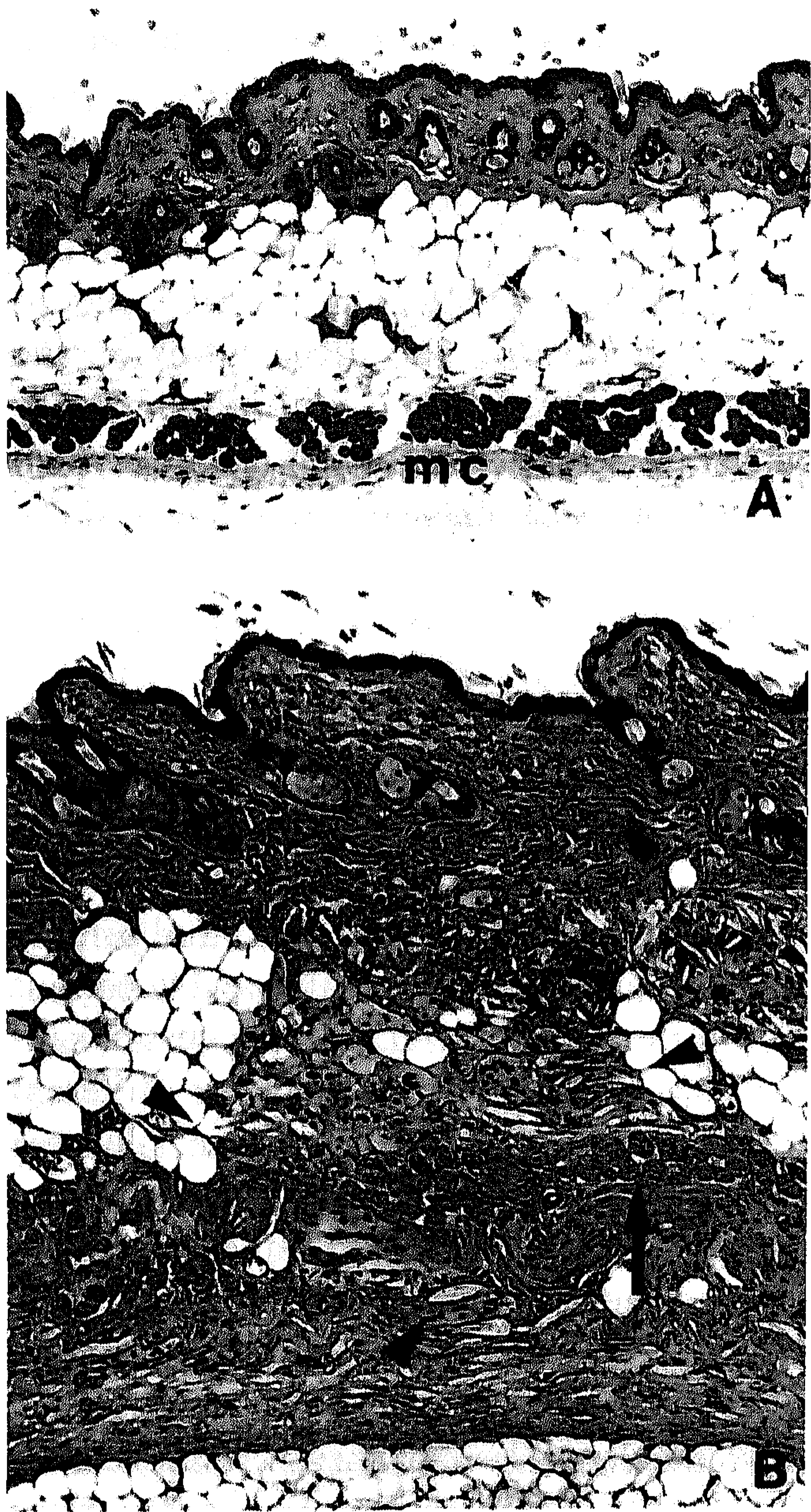
So far, all xanthomatous lesions in apo E-deficient mice were observed after histological analysis. After 14 weeks on the HFC diet, there were

no signs of macroscopic xanthoma formation. To investigate whether these mice could also develop grossly visible xanthomas, a group of 30 homozygous mutant mice and 30 wild-type littermates were fed the HFC diet for a prolonged time. About one third of the apo E-deficient mice had indeed developed xanthomas on the face and/or on the distal part of the forelimb after 6.5 months of dietary treatment, while wild-type mice did not show such abnormalities (see Fig. 2).

4. Discussion

This paper demonstrates that apo E-deficient mice fed a hypercholesterolemic diet for 14 weeks develop severe xanthomatosis. The xanthomatous lesions were identified by histological analysis, and showed a predilection for subcutaneous and peritendinous tissues, although more atypical locations were also commonly observed. The process of xanthoma formation seemed to start at different unrelated locations, such as the skin, especially near the musculus carnosus, in the peritendinous and periarticular tissues, in the submucosa of the esophagus and forestomach, and in brain and meninges. Strikingly, at the transition from the forestomach lined by squamous epithelium to the glandular stomach, the lesions abruptly stopped (see Fig. 1H): whether this intriguing finding might be due to differences in cholesterol homeostasis in the histiocytes in these two gastric mucosal locations is subject to further investigation. Many other tissues were affected, yet some major organs of the reticuloendothelial system such as liver and spleen displayed no abnormalities. Severe atherosclerotic lesions were present in the aorta and in the pulmonary and carotid arteries, as extensively reported by others [13,14].

After 14 weeks of dietary treatment, only microscopic, though extensive, xanthomatous lesions could be observed in the apo E-deficient mice, with no gross signs of xanthomatosis. Absence of gross abnormalities in *apoE* null mutants, after being fed a severe high fat diet for 3 months, was reported earlier [16]. However, we found that after feeding the mild HFC diet for 6.5 months, about one third of the apo E-deficient mice had developed xanthomas on the face and/or on the distal part of the



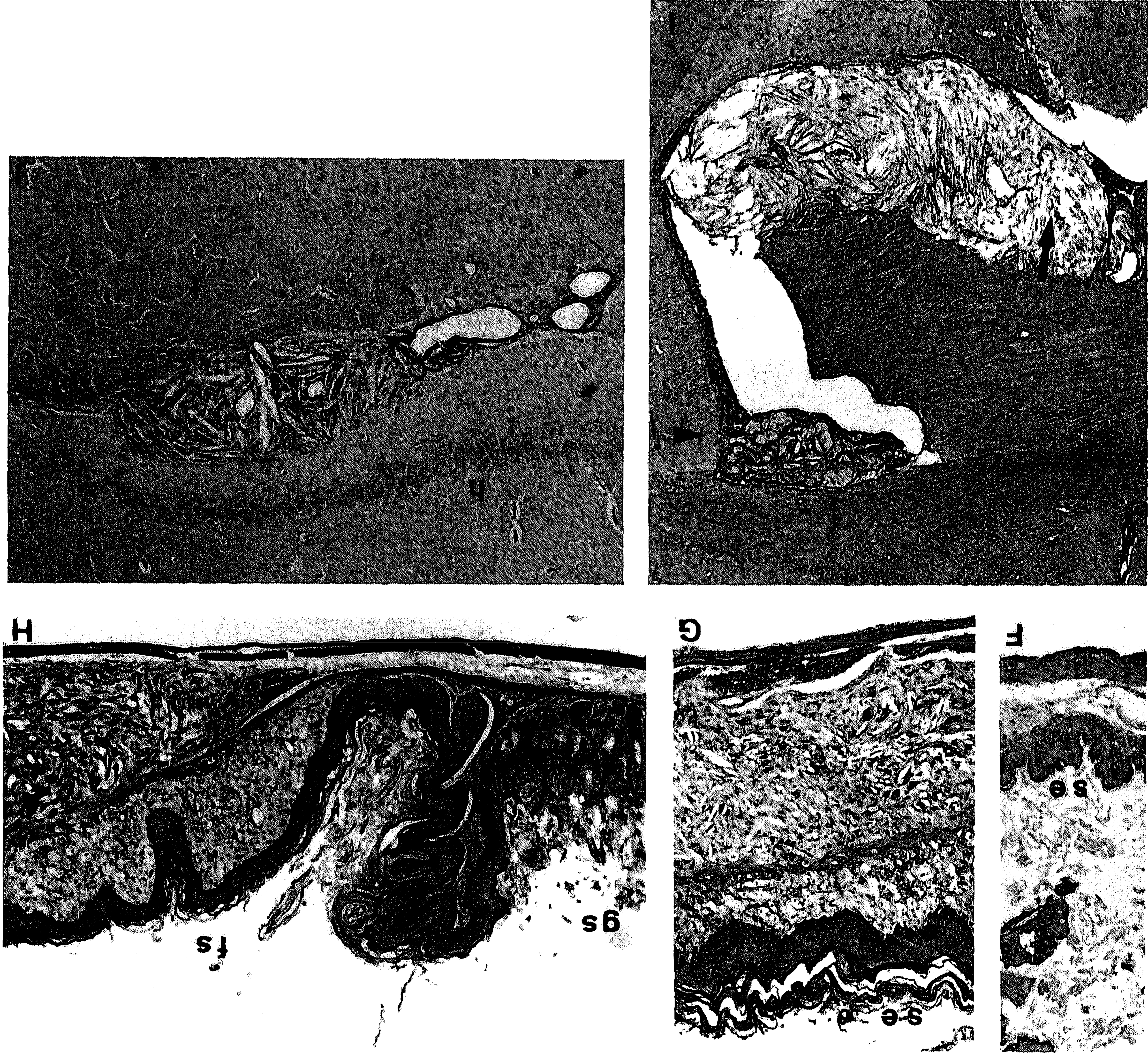


Fig. 1. Representative photomicrographs of xanthomatous lesions observed in different tissues of apoE-deficient mice. (A) Skin of a control mouse; mc, musculus carnosus; $\times 58$. (B) Early xanthomatous lesion (arrowheads) in the skin, starting near the musculus carnosus. Note remnants of the musculus carnosus (arrow), $\times 58$. (C) Severe xanthomatous lesion in the skin extending into the salivary glands (sg), $\times 58$. (D) Higher magnification of a xanthomatous lesion of the skin with cholesterol clefts (arrowheads), giant cells (short arrow) and necrosis (long arrow). This photograph is representative for xanthomatous lesions in other tissues, $\times 144$. (E) Small periarthritic and peritendinous xanthomatous lesions in the elbow (arrows), $\times 36$. (F) Forestomach of a control mouse, squamous epithelium, and peritendinous xanthomatous change in the severely thickened submucosa of the forestomach, $\times 58$. (G) Xanthomatous change in the severely thickened submucosa of the forestomach, $\times 58$. (H) Transition from forestomach (fs) to glandular stomach (gs). Note extensive accumulation of lipid-laden histiocytes in mucosa (mainly foam cells) and submucosa (with cholesterol clefts and multinucleated giant cells) of forestomach and absence of such cells in glandular stomach, $\times 58$. (I) Xanthomatous lesion at the origin of the choroid plexus (arrowheads) and subependymally in the roof of the third ventricle (arrow), $\times 36$. (J) Xanthomatous lesion in the meninges between the hippocampus (h) and thalamus (t), $\times 58$.

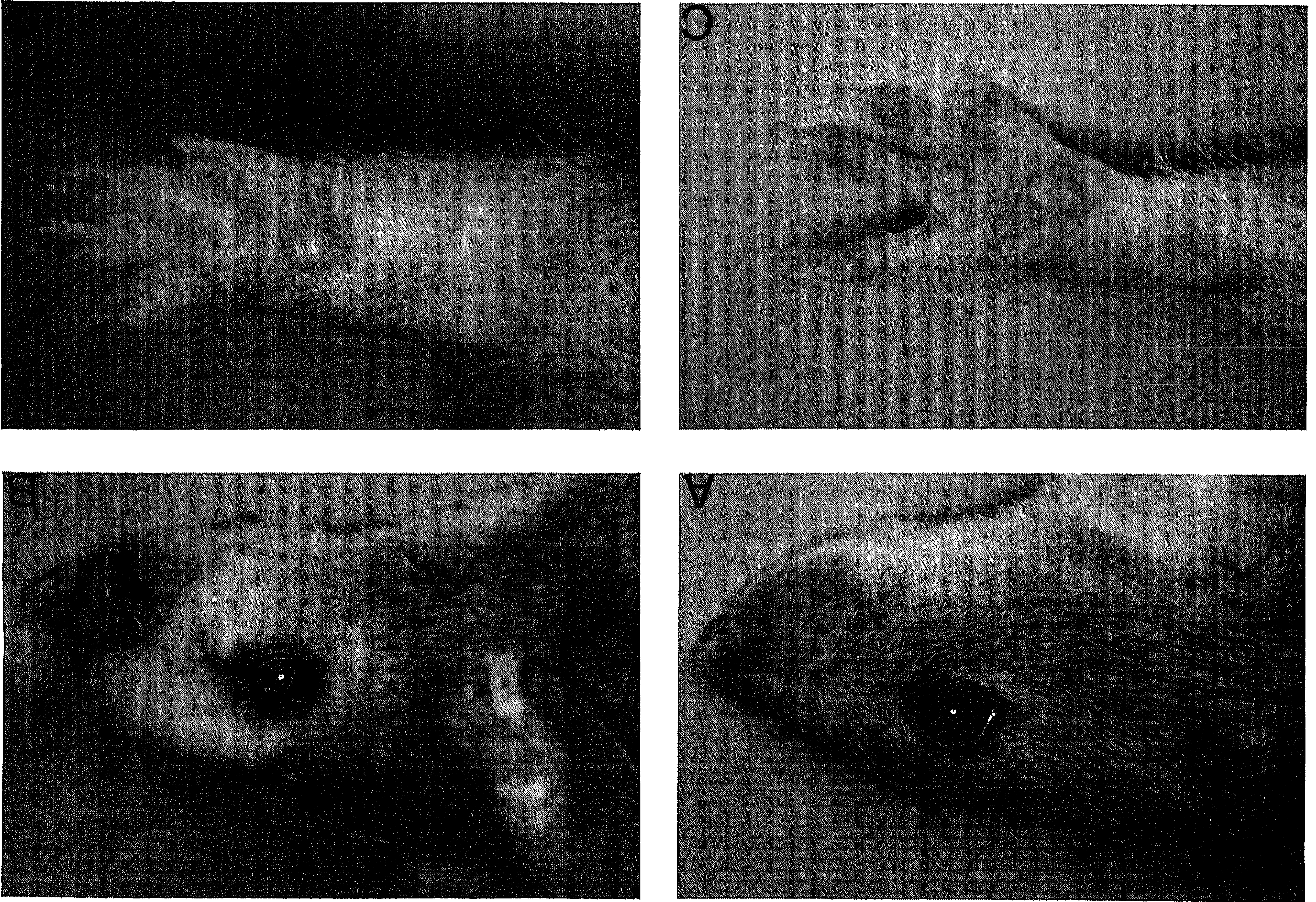


Fig. 2. Grossly visible xanthomas in an apo E-deficient mouse as compared with a wild-type littermate. Both mice were fed the HFC diet for 6.5 months, and were 7.5 months of age at the time of the photograph. (A) Face of wild-type mouse. (B) Xanthomatous lesion of face of knock-out mouse. (C) Forelimb of a wild-type mouse. (D) Xanthomatous lesion of distal part of forelimb in apoE-deficient mouse.

forelimb (Fig. 2). Although FD patients with apo E deficiency have been reported to exhibit typical tuberosus xanthomas on the elbows and xanthomata striata palmaris [4], the macroscopic xanthomas in the apo E-deficient mice do not clearly resemble these pathognomonic features. LDLR-deficient mice also showed striking macroscopic xanthomata after 7 months of cholesterol feeding, with plasma cholesterol levels comparable to our apo E-deficient mice [16]. Clearly, the rather disseminated extensive microscopic xanthomatosis long precedes the grossly visible xanthomas. Similar atypical lipid depositions have not been reported for other transgenic or knock-out mice with a disturbed lipoprotein metabolism. Therefore, we conclude that a high serum cholesterol level alone is not suf-

ficient to develop extensive microscopic xanthomatosis. For example, the apo E₃-Leiden transgenic mice displayed serum cholesterol levels up to 60 mM upon feeding a severe atherogenic diet, yet no macroscopic or microscopic xanthomas could be observed. Thus, the lack of apo E seems to be responsible for the peculiar disseminated locations of the xanthomatous lesions in the apo E-deficient mice. In other words, it seems that apo E serves an important role in determining the tissue distribution of cholesterol deposition.

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