Insulin-like Growth Factor II Plays a Central Role in Atherosclerosis in a Mouse Model*

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Insulin-like growth factor II is a fetal promoter of cell proliferation that is involved in some forms of cancer and overgrowth syndromes in humans. Here, we provide two sources of genetic evidence for a novel, pivotal role of locally produced insulin-like growth factor II in the development of atherosclerosis. First, we show that homozygosity for a disrupted insulin-like growth factor II allele in mice lacking apolipoprotein E, a widely used animal model of atherosclerosis, results in aortic lesions that are ~80% smaller and contain ~50% less proliferating cells compared with mice lacking only apolipoprotein E. Second, targeted expression of an insulin-like growth factor II transgene in smooth muscle cells, but not the mere elevation of circulating levels of the peptide, causes per se aortic focal intimal thickening. The insulin-like growth factor II transgenics presented here are the first viable mutant mice spontaneously developing intimal masses. These observations provide the first direct evidence for an atherogenic activity of insulin-like growth factor II in vivo.

Atherosclerosis is the most important cause of coronary heart disease, stroke, and peripheral arterial disease (1). The disease is characterized by the formation of intimal fibrocellular lesions consisting of smooth muscle cells (SMC), inflammatory cells, lipid deposits, and extracellular matrix in arteries of large and medium size (2). During the progression of atherosclerosis, inflammatory cells are recruited from the circulation, and SMC migrate from the underlying tissue into the lesion. Atherosclerotic lesions grow in size and complexity because of the proliferation and migration of these cell types and the deposition of extracellular matrix. Correlative and in vitro studies have implicated various growth factors in cell proliferation during atherosclerosis, but conclusive evidence for the involvement of individual molecules in vivo is lacking (1, 3, 4). The peptide insulin-like growth factor II (IGF2) is a fetal promoter of cell growth and differentiation acting mostly by autocrine or paracrine mechanisms (5, 6). IGF2 is an important regulator of body size, because mice lacking IGF2 are viable dwarfs 60% the size of wild type animals (7). IGF2 could play a major role in the growth of the atherosclerotic lesion, because it is involved in other human diseases associated with abnormal cell proliferation, such as cancer and overgrowth syndromes (8–11). Although representing an attractive candidate, IGF2 has received little attention in the study of atherosclerosis, and relatively more intense work has focused on the functionally and structurally related insulin-like growth factor 1 (IGF1) peptide (12).

In the present work, we aimed at defining the importance of IGF2 in the growth of atherosclerotic lesions in vivo. To this end, we bred mutant mice lacking apolipoprotein E (Apoe nullizygous or Apoe−/−, where − indicates a disrupted allele), a widely used mouse model with a genetic predisposition to atherosclerosis (13), that were concomitantly homozygous for a disrupted Igf2 allele (7). This cross showed how atherosclerosis develops in the absence of IGF2. We further analyzed the level of Igf2 RNA in the aortic tissue of Apoe−/− mice. In addition, we created novel mutant mice with targeted expression of an Igf2 transgene in the SMC. The latter mutation showed whether excess local IGF2 in the vascular tissue can reproduce any of the features of atherosclerosis. The contribution of these findings to elucidate the involvement of IGF2 in atherogenesis is discussed.

EXPERIMENTAL PROCEDURES

Mice with Targeted Apoe, Igf2 Alleles, and K10igf2 Transgene—Apoe nullizygous mice (generated in N. Maeda’s laboratory, University of North Carolina, Chapel Hill, North Carolina and described in Ref. 13) were purchased from M&B (Ry, Denmark) and were the tenth generation of progressive breeding on to C57BL/6J strain background. Igf2 nullizygotes were a generous gift from A. Efstradiatis (Columbia University, New York) (7) and were at >10 generations breeding on to C57BL/6J background. Double Apoe IGF2 heterozygotes obtained by outcross were intercrossed to produce the experimental genotypes: Apoe−/−, Apoe−/−/Igf2+/−, wild type (normal Apoe and Igf2 alleles), and Igf2−/−. All mice analyzed in this study were therefore in a mixed genetic background. Typing of the Apoe and Igf2 loci was performed by PCR of tail DNA with primers designed by us (details available upon request). K10igf2 heterozygous mice (K10igf2+/+) were a kind gift from C. F. Graham (University of Oxford, UK) (14) and were genotyped exactly as described (15). The crosses to obtain Apoe−/−/K10igf2+/− double mutants and control genotypes (Apoe−/−, wild type, and K10igf2+/+) were similar to the one outlined above. Starting at 8 weeks of age, mice were subject to a Western-type diet (21% total fat, 0.15% cholesterol, without sodium...
IGF2 in the Growth of Atherosclerotic Lesions

Creation of Mice with Targeted Expression of an Igf2 Transgene in SMC—A 5.1-kb Sall (blunted)-EcoRI fragment including exons 4–6 of a mouse Igf2 genomic clone (a kind gift from P. Rotwein, Oregon Health Sciences University, Portland, OR) was ligated 3' to the mouse α-smooth muscle actin gene promoter and the pSMPS (a kind gift from J. A. Fagin, University of Cincinnati, Cincinnati, OH, and A. R. Strauch, The Ohio State University, Columbus, OH; described in Ref. 16) digested with BamHI (blunted)-EcoRI following the insertion of a SpeI restriction site 5' to promoter sequences by standard targeted mutagenesis. The resulting Smaigf2 transgene was excised as an 8.7-kb SpeI-EcoRI fragment that was injected into pronuclei of F1 (C57BL/6 × NFR/N) fertilized eggs by standard techniques (17). Mice were genotyped by PCR of DNA obtained from tails using primers designed by us (details available upon request). Nine of 63 screened mice were transgenic, and lines were established from five founders. Two lines named Isac and Igor showing the two most extreme phenotypes were kept for further characterization. Transgene heterozygotes at the fourth to the sixth generations of progressive breeding on to a C57BL/6 strain background were used to analyze the growth of the aortic vessel. Mice were subjected to the Western-type dietary regime outlined above for variable periods.

Quantitation of Atherosclerotic Lesions and Tissue Morphometry—Mice were sacrificed after an overnight fast. Following microscopic dissection of the heart and aorta, photographs of the ascending aorta and aortic arch were taken with a Yashica Dental-Eye III camera (Kyocera, Tokyo, Japan). Lesions were measured in whole mount thoracic aortas and in serial sections of the ascending aorta following staining with oil red O and hematoxylin as described (19, 20). Immunohistochemistry—Primary antibodies were mouse anti-PCNA (1:750; Sigma), fluorescein isothiocyanate-conjugated anti-smooth muscle actin (1:500; Sigma), and anti-murine monoclonal/macrophages (MOMA-2 antibody; BMA Biomedicals, Augst, Switzerland). Secondary reagents designed for primary mouse antibodies used on mouse tissue (Vector, Burlingame, California) including a peroxidase-conjugated secondary antibody and a biotinylated rabbit anti-rat IgG (mouse absorbed, Vector, Burlingame, California) were employed for anti-PCNA and anti-murine monoclonal/macrophage immunohistochemistry, respectively. Nuclei expressing (brown after developing with diamobenzidine) or not expressing (blue counterstain with hematoxylin) PCNA were counted manually in printed digital images. In the case of the MOMA-2 antibody, the staining area (brown) was measured by computer-assisted morphometry in sections counterstained with hematoxylin. Controls with fluorescein isothiocyanate-conjugated mouse IgG (Dakopatts, Alvingo, Sweden) or in which the primary antibody was omitted were used for α-smooth muscle muscle actin and PCNA, respectively.

Electron Microscopy—The vessels were fixed immediately after dissection in 3% cacodylate-buffered glutaraldehyde, postfixed in 1.5% cacodylate-buffered osmium tetroxide, dehydrated in ethanol, and embedded in Spurr epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and examined in a Phillips CM120Twin electron microscope.

Detection of Endogenous and Transgene Igf2 RNA—To detect Igf2 RNA by RNase protection assay, a 277-bp template spanning the promoter-Igf2 junction in the Smaigf2 transgene including the first 15 bp of exon 2 of the α-smooth muscle actin gene and exon 4 of Igf2 and flanked by the T7 and SP6 promoters (5' and 3', respectively) was constructed by PCR (details available upon request). An Igf2 antisense probe was synthesized from the SP6 promoter, designed to produce a 167-nn (endogenous Igf2 and K10igf2 transgene) and 202-nn (Smaigf2 transgene) protected fragments (transcription reagents from Promega, Madison, WI). An antisense mouse Gapd mouse (details available upon request). An antisense probe was synthesized from the SP6 promoter designed to produce a 63-nn protected fragment, and the assay was performed as described for Igf2 RNA. The α-smooth muscle actin and Gapd RNA were quantified by analyzing a digital picture of the autoradiography film with the Kodak 1D Image analysis program (Eastman Kodak Co., Rochester, NY) for Macintosh.

Analysis of Plasma Lipid, Insulin, and Glucose—Total triglycerides and cholesterol were determined by colorimetric assays (Sigma). To measure very low density lipoprotein and high density lipoprotein, plasma was electrophoresed in a Sebia Hydragel Lipo gel (Medin Schweiz, Buochs, Switzerland), and lipids were stained with Sudan Black. Sample lipoprotein bands were scanned, and intensities were calculated relative to a human plasma reference run in the same gel. Insulin was determined by a double antibody radioimmunnoassay technique using a guinea pig anti-rat insulin antibody, 125I-labeled human insulin as tracer, rat insulin as standard, and goat anti-guinea pig IgG as secondary antibody (Linco Research, St. Charles, MO). The intra- and interassay CV was <3%. Glucose was determined with the glucose oxidase technique.

Statistics—Most of the parameters measured in this study do not unequivocally follow a normal distribution (21). Therefore, we compared matched pairs for sex and litter by the Wilcoxon paired test. Analyses were conducted using the Statview program (Abacus Concepts, Berkeley, CA) for Macintosh. Lesion sizes are reported as average with 95% confidence interval. For simplicity, the standard deviation is used in all other cases. The letter n indicates the number of pairs analyzed, unless stated otherwise.

RESULTS

Reduced Atherosclerosis in Apoe−/− Mice Lacking IGF2—A gross inspection of the ascending aorta revealed that 18-week-old Apoe−/− mice subject to a Western-type diet for 10 weeks developed lesions at the origin, the lesser curvature of the arch, and the branching points as previously reported (Fig. 1A and Ref. 22). By comparison, the corresponding lesions in mice with

FIG. 1. The lack of IGF2 reduces atherosclerosis in Apoe nullizygous mice. A and B, gross inspection of the ascending aorta and aortic arch of Apoe−/− (A) and Apoe−/−/Igf2−/− (B) mice. The arrowheads show lesions at the aortic origin, lesser curvature of the arch, and branching points. C–H, whole mount aortas of Apoe−/− (C and D, in duplicate), Apoe−/−/Igf2−/− (E and F, in duplicate), wild type (G), and Igf2−/− (H) mice. The arrowheads and arrows show lesions stained in dark red at the insertion of intercostal arteries and luminal wall, respectively. The areas stained in light red represent adventitial fat. Original magnification, ×20. Bar, 0.5 mm.
disruption of both Apoe and Igf2 alleles (Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup>) were fewer and smaller (Fig. 1B). We measured the size of lesions at two different sites, e.g. the ascending and the thoracic aorta, by the inspection of serial sections and whole mount aortas stained with oil red O, respectively. The total surface of atherosclerotic lesions in whole mount thoracic aortas of Apoe<sup>−/−</sup> mice was 0.47 mm<sup>2</sup> (95% confidence interval (CI) = 0.30–0.64, n = 14). Fatty lesions were detectable at branching points and on the luminal wall in Apoe<sup>−/−</sup> mice (Fig. 1, C and D, in duplicate). By contrast, the size of lesions in whole mount thoracic aortas of Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice was 0.09 mm<sup>2</sup> (CI = 0.04–0.13, n = 14; Fig. 1, E and F, in duplicate), amounting to a nearly 80% reduction compared with Apoe<sup>−/−</sup> (p = 0.0077). No lesion was detectable in aortas of Igf2 nullizygous (Igf2<sup>/−</sup>) or wild type mice (n = 7 and 8, respectively; Fig. 1, G and H). A similar reduction of atherosclerosis in Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice was revealed by a quantitative analysis of serial sections of an 800-μm-long segment of the ascending aorta. The average lesion area was 0.16 mm<sup>2</sup> (CI = 0.11–0.30) in Apoe<sup>−/−</sup> and 0.02 mm<sup>2</sup> (CI = 0.005–0.05) in Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice (p = 0.0201, n = 10).

Mice lacking IGF2 are dwarfs with organs variably reduced in size (compare G with H in Fig. 1; Refs. 7, 15, and 23). The reduced size of the aorta in dwarf mice lacking IGF2 could be a factor contributing to the decrease in lesion size observed in Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice. Nevertheless, the size of lesions in both the thoracic and the ascending aorta was significantly different in Apoe<sup>−/−</sup> and Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice even after adjusting for the size of the vessel (Fig. 2) or body weight (not shown).

**Morphology and Composition of Lesions**—The histopathology of lesions in sections of the ascending aorta was qualitatively similar in Apoe<sup>−/−</sup> and Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice. Both groups developed intimal fibrocellular lesions containing lipid-rich cores and a layer of fibromuscular tissue on the luminal aspect. Lesions with a similar morphology were previously described in Apoe<sup>−/−</sup> mice (21).

The reduced lesion size in Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice may be due to a decreased cell proliferation or migration of SMC, because both processes are stimulated by IGF2 in vitro (5, 24). We studied the rate of cell proliferation and the abundance of SMC within lesions of Apoe<sup>−/−</sup> and Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice by immunohistochemistry with antibodies specific for the PCNA and α-smooth muscle actin, respectively. In the case of Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice, only sections with relatively large lesions containing a sufficient number of cells to allow meaningful comparisons were included in the histological analysis. The frequency of cells expressing PCNA in lesions of Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice was 22.6 ± 7.6% or nearly half the value measured in lesions of Apoe<sup>−/−</sup> mice (39.5 ± 5.9%, n = 6, p = 0.02). Cells expressing α-smooth muscle actin were abundant and densely packed around lipid-rich cores in Apoe<sup>−/−</sup> lesions (Fig. 3, A and C). By contrast, α-smooth muscle actin was less abundant and present in sparsely distributed cells in Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> lesions (Fig. 3, B and D). Although the staining in the media was generally weak, the fluorescence observed in lesions originated from genuine staining of α-smooth muscle actin, as shown by the control in which the fluorescent antibody was omitted (inset in upper left corner of Fig. 3A). The α-smooth muscle actin was specifically decreased in lesions of Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice, whereas the medial tissue, which represents the majority of aortic SMC in the mice included in the present study (19), contained apparently normal levels of the polypeptide (Fig. 3B). Accordingly, RNase protection analysis of aortic RNA revealed only a slight reduction (~5%) in α-smooth muscle actin transcript in Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> compared with Apoe<sup>−/−</sup> mice (not shown). Preliminary results showed that monocytes/macrophages were abundant in lesions from both Apoe<sup>−/−</sup> and Apoe<sup>−/−</sup> / Igf2<sup>−/−</sup> mice, with little difference between the two groups (not shown).

**IGF2 Acts as an Autocrine or Paracrine Factor in Atherosclerosis**—The observation that IGF2 transgenes have mainly local effects (5, 14, 25) suggests that IGF2 may promote cell proliferation in atherosclerotic lesions by autocrine or paracrine
mechanisms. Accordingly, we detected Igf2 RNA in aortas of eight of ten Apoe−/− mice tested by RNase protection assay or reverse transcription-PCR (Fig. 4). This variability may reflect the broad distribution of lesion size (Fig. 2). Igf2 RNA generally declines to nearly undetectable levels in murine adult tissues and was not detected in wild type aortas (Fig. 4; n = 4).

Furthermore, to study the effects of locally produced IGF2 on vascular cell proliferation, we created mice with a persistent supply of the peptide in blood vessels throughout adult life by targeted expression of an Igf2 transgene in SMC (Smaigf2 transgene; Fig. 4). The mouse α-actin smooth muscle promoter included in the Smaigf2 transgene has been previously proven to correctly target transgene expression to the SMC (16, 26). The size of the aorta and other organs rich in SMC was increased in transgenics belonging to two independent lines (Igor and Isac lines; Fig. 5, A and B). These effects were autocrine or paracrine, because Smaigf2 heterozygotes did not show any significant deviation from matched wild type mice in either body length (p = 0.136, n = 6 in the Isac line; p = 0.257, n = 6 in the Igor line) or tibial length (p = 0.102, n = 6, Isac line; p = 0.785, n = 6, Igor line) or wet weight of organs in which SMC are a relatively minor component, such as the tongue (p = 0.893, n = 7, Isac line; p = 0.50, n = 7, Igor line). These observations suggest that the increase in body weight observed in Smaigf2 transgenics compared with wild type mice (Isac line: +22.6%, p = 0.102, n = 6; Igor line: +12.5%, p = 0.785, n = 6) was due to the disproportionate growth of organs rich in SMC. In addition, Smaigf2 heterozygotes belonging to both lines developed aortic focal thickenings that were present from the earliest age studied (8 weeks) on and were independent of dietary regime (n = 7; Fig. 5, B and C). No thickening was detected in any of the matched wild type aortas examined in this or other studies conducted in our laboratory (n = 15; Fig. 5A). Electron microscopy studies showed that these thickenings were intimal, consisted exclusively of contractile SMC, and contained no lipid deposits (Fig. 6A; two randomly chosen sites on the aortic arch were sectioned, and four or five sections/site were examined in three Smaigf2 heterozygotes). Leukocytes adhering to the endothelial surface have been observed in only one section. By contrast, the intima of matched wild type littersmates was normal, e.g. consisted of a monolayer of endothelial cells and lacked any SMC (Fig. 6B; n = 3). No obvious difference in the phenotype of medial SMC between wild type and Smaigf2 mice was detectable by electron microscopy. Smaigf2 heterozygotes had fasting plasma levels of cholesterol and triglycerides similar to wild type mice (not shown).

Although the levels of circulating IGF2 in Smaigf2 transgenics are not known, the phenotype may be caused by the endocrine activity of an excess systemic transgene IGF2 leaking from expressing cells. To clarify the importance of systemic IGF2, we exploited mice with elevated circulating IGF2 as a result of expression of an Igf2 transgene controlled by the keratin 10 promoter (K10igf2 transgene; Ref. 14). Aortas of adult K10igf2 heterozygous mice had no detectable Igf2 RNA and showed no signs of intimal thickenings (n = 7; not shown).
Accordingly, the growth of atherosclerotic plaques was not significantly increased by circulating IGF2, because mice that were Apoε−/− and K10igf2 heterozygous had lesions in the thoracic aorta of a size similar to Apoε single nullizygotes (0.53 mm²; CI = 0.30–0.77; n = 12; p = 0.8655 in comparison with Apoε−/− mice).

Reduced Hyperlipidemia in Apoε−/− Mice Lacking IGF2—As previously reported, Apoε−/− mice showed hypercholesterolemia, a mild hypertriglyceridemia, an increase in very low density lipoprotein, and a decrease in high density lipoprotein (Table I and Ref. 13). The absence of IGF2 ameliorated this dyslipidaemic profile. Fasting total cholesterol and very low density lipoprotein were reduced by 45 and 35%, respectively, in Apoε−/−/Igf2−/− compared with Apoε−/− mice, and total triglycerides were at levels not significantly different from wild type (Table I). High density lipoprotein showed a tendency to increase in Apoε−/−/Igf2−/− mice, although the difference with Apoε−/− was not significant (Table I). Thus, IGF2 significantly contributes to elevating plasma lipids in Apoε−/− mice. In addition, fasting plasma insulin levels were normal in Apoε−/− mice as previously reported (22) but were nearly doubled and inversely correlated with triglyceride levels in Apoε−/−/Igf2−/− mice (Table I; r = −0.833; p = 0.02; n = 7). The compensatory elevation of insulin levels might then underlie the reduced hyperlipidemia in Apoε−/−/Igf2−/− mice. This increase in insulin levels was not a consequence of the lack of IGF2 per se, because Igf2−/− mice were normoinsulinaemic as reported (Table I and Ref. 27), but was rather due to the reactivation of compensatory adjustments of insulin levels that were inhibited by IGF2. Fasting glucose levels were normal in all genotypes analyzed (Table I). The latter observation suggests that the increase in insulin secretion observed in Apoε−/−/Igf2−/− mice was concomitant with a reduced sensitivity of circulating glucose to insulin.

**DISCUSSION**

Our work expands the current knowledge of the role of IGF2 in disease. It provides the first genetic evidence that IGF2 is a major promoter of growth of atherosclerotic lesions and a factor contributing to dyslipidaemia in mice lacking apolipoprotein E, a widely used animal model with genetic predisposition to atherosclerosis and hyperlipidemia. IGF2 increases the size of atherosclerotic lesions by stimulating cell proliferation. The effects of IGF2 on the migration of SMC are less clear. The decrease in α-smooth muscle actin observed in Apoε−/−/Igf2−/− lesions may reflect a combination of reduced migration and phenotypic change of SMC. Indeed, experiments with cultured SMC showed that IGF2 prolongs the expression of markers of the contractile phenotype, which include α-smooth muscle actin (28).

The effects of IGF2 on the levels of circulating lipids are difficult to explain based on the current knowledge of the metabolic activity of this growth factor (29). On the other hand, a significant positive correlation between plasma levels of IGF2 and total cholesterol has been observed in diabetic patients (30). Our data suggest that IGF2 may maintain hyperlipidemia in Apoε−/− mice by inhibiting compensatory adjustments of insulin levels. Indeed, a negative control of insulin levels by IGFs has been reported in humans following administration of recombinant IGF1 and in mice expressing high levels of an Igf2 transgene driven by the major urinary protein promoter (31–33). Although reproducing some of our observations, these studies are difficult to interpret, because an increase in IGF1 is associated in some cases with a better glycaemic control by insulin, and hyperinsulinaemia has been observed in mice expressing an Igf2 transgene in the pancreas (33, 34). These discrepancies suggest that the net metabolic effect of the lack of IGF2 in Apoε−/− mice is the sum of profoundly different organ-specific local responses.

Three independent lines of evidence strongly suggest that IGF2 promotes the proliferation of intimal cells during atherosclerosis by an autocrine or paracrine mechanism. First, Igf2 RNA is present in aortas of Apoε−/− but not wild type mice. Second, the increased local supply of IGF2 in SMC per se results in the formation of aortic focal intimal cushions. Third, mice with elevated circulating IGF2 but no peptide produced in the aortic vessel do not show any intimal cushions nor have increased lesion size in an Apoε−/− background. These local effects are probably synergistic with systemic changes in circulating atherogenic lipids. To better understand the interplay between IGF2 and atherogenic lipids in the development of fatty lesions, we are currently breeding the Smaigf2 transgene on to an Apoε−/− background. If IGF2 acts mainly locally, mice that are Smaigf2 heterozygous and Apoε−/− are predicted to show an increase in lesion size with
few metabolic changes.

The development of intimal masses in Smad5 transgenic mice is an unexpected and potentially important curiosity. A muscular intimal layer is characteristically absent from murine vessels and can develop as a consequence of vascular cuff formation on the meaning of intimal cushions and for reading the manuscript, the personnel of the animal facility at the Experimental Department, Malmö General Hospital, Malmö for excellent assistance. Kivist for continuous enthusiasm and help, and M.A. Sallstrom of the Lund Transgenic Core Facility for skillfully performing embryo transfers between distant cities.

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The values are expressed as the averages ± S.D. n is the number of observations. VLDL, very low density lipoprotein; HDL, high density lipoprotein.

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<tr>
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<th>Apo e/–</th>
<th>Apo e/–/Igf2 e/–</th>
<th>Wild type</th>
<th>Igf2 e/–</th>
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<tr>
<td>Cholesterol (mg/ mol)</td>
<td>6.1 ± 0.7 (n = 22)</td>
<td>3.6 ± 0.4 (n = 11 p,b,c)</td>
<td>0.7 ± 0.1 (n = 10 a)</td>
<td>0.5 ± 0.1 (n = 5 a)</td>
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<tr>
<td>Triglycerides (mg/ml)</td>
<td>1.9 ± 0.3 (n = 22)</td>
<td>1.0 ± 0.2 (n = 12 a)</td>
<td>0.7 ± 0.1 (n = 13 a)</td>
<td>0.6 ± 0.1 (n = 5 a)</td>
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<td>VLDL (arbitrary units)</td>
<td>1.53 ± 0.19 (n = 21)</td>
<td>1.05 ± 0.12 (n = 10 p,b,c)</td>
<td>0.19 ± 0.04 (n = 11 a)</td>
<td>0.21 ± 0.02 (n = 4 a)</td>
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<tr>
<td>HDL (arbitrary units)</td>
<td>0.16 ± 0.02 (n = 21)</td>
<td>0.19 ± 0.03 (n = 10 p,b,c)</td>
<td>0.51 ± 0.05 (n = 11 a)</td>
<td>0.30 ± 0.04 (n = 4 a)</td>
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<tr>
<td>Insulin (pmol/L)</td>
<td>38.3 ± 16.3 (n = 20)</td>
<td>76.1 ± 19.3 (n = 7 p,b,c)</td>
<td>24.1 ± 10.1 (n = 10)</td>
<td>33.3 ± 13.3 (n = 4)</td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>3.9 ± 0.6 (n = 20)</td>
<td>4.2 ± 0.4 (n = 7)</td>
<td>4.1 ± 0.4 (n = 10)</td>
<td>3.1 ± 0.2 (n = 4)</td>
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* Significant difference (p < 0.05) from Apo e/–.

b Significant difference (p < 0.05) from wild type.

c Significant difference (p < 0.05) from Igf2 e/–.

TABLE I

Fasting plasma lipids, insulin, and glucose

REFERENCES

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