

Abbreviation and symbols GI = gastrointestinal, x = not recommended, 3 = recommended, ! = use with caution,
- = insufficient data to make a recommendation

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pertenecen al estado de la técnica desde antes de la fecha de prioridad de la presente solicitud

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II. COMPLEMENTACIÓN DE LA INFORMACIÓN

Llamo la atención al despacho sobre la relevancia de los argumentos que se enuncian a continuación

1) La publicación titulada "Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis" de NATURE MEDICINE¹, revela que la proteína aP2 se encuentra originalmente en el tejido adiposo. En estudios realizados en ratones con deficiencia de aP2, muestran que la pérdida de ésta proteína tiene un fuerte impacto en aspectos relacionados con el síndrome metabólico. La deficiencia de aP2 proporciona una protección significativa frente a la hiperinsulinemia y la resistencia a la insulina, asociada con la obesidad dietaria o genética. Así mismo, la presencia de aP2 contribuye a elevar el nivel de glucosa sistémica y el metabolismo lipídico, y altera la cantidad de lipólisis de adipocitos. Como la resistencia a la insulina y el metabolismo anormal de lípidos son factores de riesgo para enfermedades cardiovasculares, es posible que aP2 influya en el desarrollo de la aterosclerosis.

Lo anterior, con el ánimo de hacer notar que la proteína aP2 había sido revelada con anterioridad en el estado de la técnica, y era conocida su influencia en la elevación de los niveles de glucosa sistémica y metabolismo lipídico, lo cual, como es sabido, contribuye al desarrollo de la aterosclerosis. En consecuencia, si la presencia de la proteína aP2 desarrolla algunas patologías, es obvio para una persona versada en la materia, que la estrategia farmacológica adecuada sea no sólo administrar inhibidores de aP2 -que como su nombre lo indica inhibe la acción de la proteína-, sino administrarlo en combinación con otros agentes para potenciar su efecto tal como lo pretende la solicitud colombiana CO-00-024-081.

2) La solicitud en cuestión en la Reivindicación 20, pretende proteger una composición farmacéutica que comprende un compuesto inhibidor de aP2 y un agente antidiabético, donde este último puede ser 1, 2, 3 o más de biguanida, sulfonilurea, inhibidores de glucosidasa y/o meglitinida, entre otros.

La combinación de un inhibidor de aP2 con otro tipo de los mencionados en dicha reivindicación, pretende hacer que el efecto contra la diabetes, la obesidad, la disminución

¹ NATURE PUBLISHING GROUP. Aunque el artículo fue publicado en Junio de 2001, una gran cantidad de los documentos citados en la bibliografía fueron publicados con anterioridad a la fecha de publicación de la prioridad de la solicitud CO-00-024-081.

de lípidos, sea mayor que aquel que se obtendría mediante la administración individual de cada uno de los componentes

Sobre este respecto, hago notar que existen un gran número de publicaciones que revelan combinaciones que contienen agentes antidiabéticos. Para ilustración de ese Despacho, me permito anexar a este escrito, un documento publicado por la Universidad de Pamplona titulado *"Tratamiento combinado de la diabetes tipo 2"* de E. Menéndez² que demuestra la posibilidad de realizar terapias combinadas con la presencia de agentes antidiabéticos. En la página 2 del documento, consta la posibilidad de combinar biguanidas con sulfonilureas donde las biguanidas disminuyen la excesiva producción hepática de glucosa. Por lo tanto, es obvio que haciendo una combinación de los agentes mencionados con inhibidores de $\alpha P2$, se potenciaría el resultado para tratar enfermedades de diabetes.

En el mismo sentido, la reivindicación 21 establece que el agente antidiabético es 1, 2, 3, o más de gliburida, glimepirida, insulina y repaglinida y/o nateglinida. La página 2 del mismo escrito, revela la posibilidad de adicionar una biguanida -metformina en ese caso- a la terapia de los pacientes tratados con sulfonilureas, repaglinida o nateglinida, disminuyendo la HbA1c (hemoglobina glicosilada) de un 1,5% a un 2,2%. Lo anterior demuestra, una vez más, la falta de nivel inventivo de la solicitud en cuestión.

3) El documento *"New Oral Antihyperglycemic Agents Expand Armamentarium in the Battle Against Type 2 Diabetes Mellitus"*³ - el cual se anexa a este escrito- fue publicado en 1998 y revela los efectos de combinar diferentes agentes anti-hiperglucemiantes con otros agentes antidiabéticos en pacientes con la Diabetes Mellitus Tipo 2. Llamo especialmente la atención a ese Despacho sobre la pretensión de la Reivindicación 21, de proteger una composición donde el agente antidiabético puede ser la troglitazona. El documento antes mencionado muestra claramente la posibilidad de combinar troglitazona con otro agente antidiabético, siendo ésta una combinación comúnmente utilizada.

4) La reivindicación 21 revela una combinación caracterizada porque el agente antidiabético puede ser GI-262570. Llamo la atención al Despacho, que esa entidad en Concepto No. 678 de abril de 2000, folio 323, manifestó que en las reivindicaciones de la solicitud, no eran aceptables como nombre de las sustancias, un código o una referencia numérica como GI-252670, porque químicamente no significaban nada ni hacían referencia a ninguna sustancia.

² www.cfnava.es. Aunque no se conoce la fecha de publicación del documento anexo, la gran mayoría de los escritos que se citan como bibliografía, fueron publicados con anterioridad a la fecha de prioridad de la solicitud colombiana CO-00-024-081.

³ <http://www.medscape.com>. "New Oral Antihyperglycemic Agents Expand Armamentarium in the Battle Against Type 2 Diabetes Mellitus" 1998.

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Además de las solicitadas en el escrito de oposición, y de aquellas que oficiosamente considere el despacho, ruego que se tengan como tales las siguientes

3 1 Copia parcial del artículo "Tratamiento combinado de la diabetes tipo 2" de E Menéndez de la Universidad de Pamplona

3 2 Copia íntegra del artículo "New Oral Antihyperglycemic Agents Expand Armamentarium in the Battle Against Type 2 Diabetes Mellitus" publicado en 1998

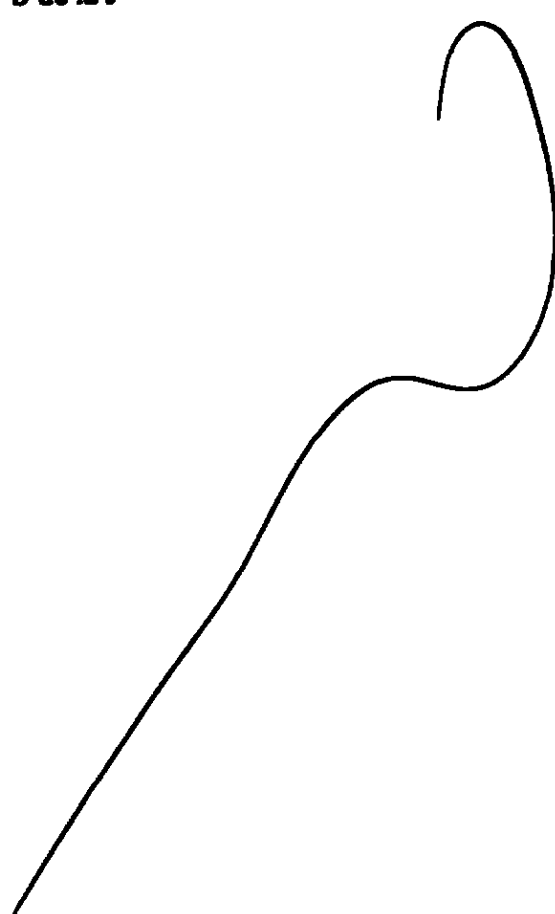
3 3 Copia íntegra del artículo "Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis" de NATURE MEDICINE- volumen 7- No 6 de junio de 2001

Señor Superintendente,

JOSE LUIS REYES VILLAMIZAR

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Lack of macrophage fatty-acid-binding protein aP2 protects mice deficient in apolipoprotein E against atherosclerosis

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The adipocyte fatty-acid-binding protein, aP2, has an important role in regulating systemic insulin resistance and lipid metabolism. Here we demonstrate that aP2 is also expressed in macrophages, has a significant role in their biological responses and contributes to the development of atherosclerosis. Apolipoprotein E (ApoE)-deficient mice also deficient for aP2 showed protection from atherosclerosis in the absence of significant differences in serum lipids or insulin sensitivity. aP2-deficient macrophages showed alterations in inflammatory cytokine production and a reduced ability to accumulate cholesterol esters when exposed to modified lipoproteins. *ApoE*^{−/−} mice with *ApoE*^{−/−} adipocytes and *ApoE*^{−/−} macrophages generated by bone-marrow transplantation showed a comparable reduction in atherosclerotic lesions to those with total aP2 deficiency, indicating an independent role for macrophage aP2 in atherogenesis. Through its distinct actions in adipocytes and macrophages, aP2 provides a link between features of the metabolic syndrome and could be a new therapeutic target for the prevention of atherosclerosis.

Adipocyte fatty-acid-binding protein, aP2 (encoded by *ApoB*), is a member of the intracellular fatty-acid-binding protein (FABP) family. Cytoplasmic FABPs are small proteins that are expressed in a highly tissue-specific manner and bind to fatty acids^{1,2}. aP2 is primarily detected in adipose tissue and its expression is highly regulated during differentiation of adipocytes^{3,4}. Moreover, expression of *ApoB* mRNA is transcriptionally controlled by fatty acids^{5,6}. Recent studies in aP2-deficient mice have shown that loss of this protein has a critical impact on several aspects of the metabolic syndrome. First, lack of aP2 provides significant protection from hyperinsulinaemia and insulin resistance associated with dietary or genetic obesity^{7,8}. Second, aP2 contributes to improved systemic glucose and lipid metabolism in the setting of dietary or genetic obesity^{9,10} and alters the rate of adipocyte lysis^{11,12}. As both insulin resistance and abnormal lipid metabolism are risk factors for cardiovascular disease, it is possible that aP2 influences the development of atherosclerosis by modulating these factors.

Several lines of evidence indicate a striking overlap between the biology of adipocytes and macrophages. Genes that are critical in adipocytes, including those encoding transcription factors, cytokines, inflammatory molecules, fatty acid transporters and scavenger receptors, are also expressed in macrophages and have an important role in their biology¹³. For example, peroxi-

some-proliferator activated receptor-γ (PPAR-γ) is a member of the nuclear-receptor superfamily of ligand-activated transcription factors that regulates adipocyte development and glucose homeostasis. PPAR-γ is also expressed in activated monocyte/macrophages and might have a role in generating the inflammatory response and forming the foam cells associated with atherosclerotic lesions^{14,15}; however, the mechanisms for these functions are unclear. Interestingly, aP2 expression was recently revealed in human monocytes following stimulation with PPAR-γ activators¹⁶, and oxidized low-density lipoprotein has been reported to induce expression of aP2 in human THP-1 macrophages¹⁷. These observations indicate that expression of aP2 by macrophages might also influence foam-cell formation and thereby atherosclerotic processes, possibly through mechanisms independent of its metabolic effects.

Here we examined whether macrophage aP2 influences foam-cell formation and the development of atherosclerosis independent of the effects of aP2 on insulin resistance and plasma lipids. Using two different models, *ApoE*^{−/−} mice intercrossed with *ApoE*^{−/−} mice and the bone-marrow transplantation (BMT) model in which *ApoE*^{−/−} mice are donors to recipient *ApoE*^{−/−} mice, we demonstrate that aP2-deficient macrophages provide significant protection against atherosclerosis in the ApoE-deficient model in the absence of differences in glucose and lipid metabolism.

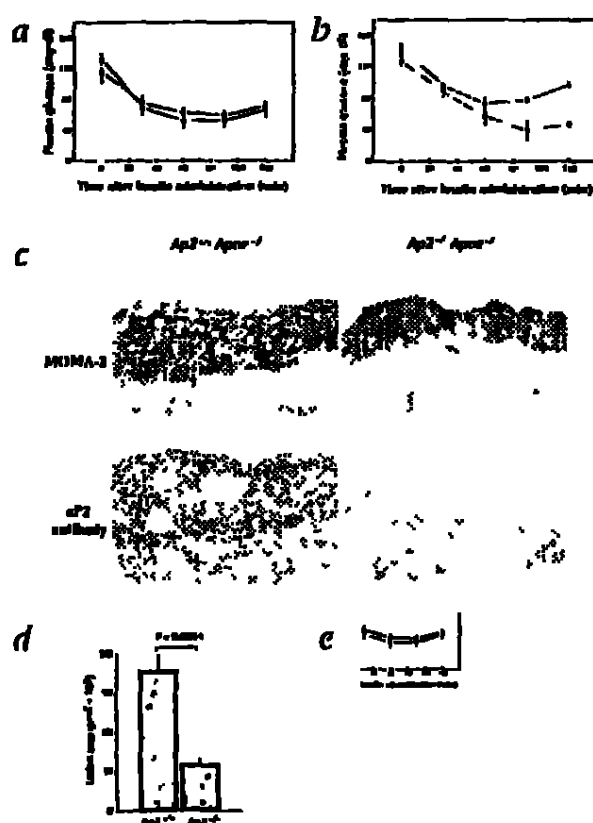
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Fig 1 Insulin sensitivity and atherosclerosis in $Ap2^{+/+}Apoe^{-/-}$ and $Ap2^{-/-}Apoe^{-/-}$ mice on chow diet. **a** and **b**, Insulin tolerance tests were performed in 15 male (**a**) and 7 female (**b**) 14-week-old $Apoe^{-/-}$ (Δ) and $Ap2^{+/+}Apoe^{-/-}$ ($\square$), mice. Data are mean $\pm$ s.e.m., $P < 0.05$. **c**, Immunocytochemical detection of macrophages and ap2 expression in the proximal aorta of $Ap2^{+/+}Apoe^{-/-}$ (left) and $Ap2^{-/-}Apoe^{-/-}$ (right) mice. Macrophages are stained with MOMA-2 (upper), and ap2 is detected with polyclonal rabbit antiserum against mouse ap2 (lower). **d** and **e**, Quantification of atherosclerotic lesion area in the proximal (**d**) and en face (**e**) in $Ap2^{+/+}Apoe^{-/-}$ and $Ap2^{-/-}Apoe^{-/-}$ mice on chow diet. Data are represented as the average mean lesion area for each group.

All of the mice developed moderate, fatty streak lesions, consisting predominantly of macrophage-derived foam cells, as determined by immunocytochemistry using the nonreactive antibody against mouse macrophages²¹, MOMA-2 (Fig. 1c, upper panels). The macrophage-derived foam cells stained strongly positive for ap2 in the lesions of the $Ap2^{+/+}Apoe^{-/-}$ but not in the $Ap2^{-/-}Apoe^{-/-}$ mice (Fig. 1c, lower panels). Quantitative analysis of the proximal aorta revealed that the mean atherosclerotic lesion area in $Ap2^{+/+}Apoe^{-/-}$ males was reduced by 66% compared with $Ap2^{-/-}Apoe^{-/-}$ males ($16,314 \pm 3,492$ versus $107,815 \pm 11,144$, μm^2 /section $\pm$ s.e.m., $P < 0.0014$, Fig. 1d). A similar reduction (87.6%) was found by en face analysis of the extent of atherosclerosis in the entire aorta in $Ap2^{+/+}Apoe^{-/-}$ males compared with $Ap2^{-/-}Apoe^{-/-}$ controls (0.60 ± 0.15 versus 0.074 ± 0.02 , percent $\pm$ s.e.m., $P < 0.005$, Fig. 1e). Thus, in the setting of a normal chow diet, male $Apoe^{-/-}$ mice lacking ap2 were protected from the development of macrophage-derived foam cells and atherosclerosis. In a separate experiment, the extent of atherosclerosis in the proximal aorta was reduced by 75% in male and by 26% in female $Ap2^{+/+}Apoe^{-/-}$ mice fed the Western diet for 14 weeks compared with $Ap2^{+/+}Apoe^{-/-}$ controls ($P < 0.0024$ and $P < 0.006$, respectively; data not shown).

FABP expression and function in macrophages

Given that atherosclerotic lesions showed strong presence of ap2 and the differences in atherosclerosis occurred in the absence of significant metabolic alterations, macrophage ap2 expression might be responsible for the observed differences in atherosclerosis. We therefore investigated ap2 expression and regulation in primary isolated macrophages and macrophage cell lines. In both THP-1 and U-937 human monocyte/macrophage cell lines, we did not detect ap2 expression in resting cells but did so after stimulation by PMA (phorbol 12-myristate 13-acetate) to induce differentiation (Fig. 2a and b). Similarly, primary human monocytes did not express ap2 protein, but we observed high levels upon stimulation with PMA (Fig. 2d). In contrast, we did not detect ap2 expression by northern blot analysis of resting or stimulated HLA-1-cell or M12 B-cell lines (data not shown). The keratinocyte FABP, mal1, which is the second isoform expressed in adipocytes, was also present in these cells and was regulated in an essentially identical manner upon PMA stimulation, although low levels were detectable in the resting state in THP-1 and U-937 cell lines (Fig. 2a and b). Expression of both ap2 and mal1 was also observed in isolated peritoneal mouse macrophages. Unlike the compensatory regulation in adipocytes, mRNA encoding mal1 did not appear to be significantly upregulated in $Ap2^{+/+}$ primary macrophages (Fig. 2c). We then investigated whether the regulatory elements that direct expression of ap2 in adipocytes are sufficient to confer expression in macrophages of transgenic mice. For this we used three independent lines expressing genes encoding uncoupling protein (UCP)-1,

Metabolism and atherosclerosis in $Ap2^{+/+}Apoe^{-/-}$ mice

Feeding C57BL/6 mice a high-fat diet promotes the development of obesity and insulin resistance²², which could impact the development of atherosclerosis²³. We therefore initially used $Apoe^{-/-}$ mice, which on a normal chow diet develop spontaneous atherosclerotic lesions that are largely independent of obesity, hyperglycemia and hyperinsulinemia. To analyze the role of ap2 in atherosclerotic lesion formation, we intercrossed $Ap2^{+/+}$ mice with $Apoe^{-/-}$ mice. At 4 weeks, we divided littermates into an experimental group of $Ap2^{+/+}Apoe^{-/-}$ ($n = 7$) and a control group of $Ap2^{-/-}Apoe^{-/-}$ ($n = 7$) mice and fed them a regular chow diet ($< 4.5\%$ fat) for 14 weeks. After insulin tolerance tests and steady-state biochemical measurements, we analyzed the extent of aortic atherosclerosis at 18 weeks. After a four-hour fast, total serum cholesterol (445 ± 36 versus 478 ± 12 , mg/dl $\pm$ s.e.m.) and triglyceride (142 ± 27 versus 191 ± 17 , mg/dl $\pm$ s.e.m.) levels were elevated as a result of $Apoe^{-/-}$ deficiency in both $Ap2^{+/+}$ and $Ap2^{-/-}$ groups, respectively, without significant differences between genotypes. In addition, serum glucose levels did not differ significantly between the experimental and control mice (117 ± 5.9 and 126.3 ± 17 , mg/dl $\pm$ s.e.m., $P = 0.53$). There was a reduction in total cholesterol, triglyceride and glucose, but not insulin levels in the $Ap2^{+/+}Apoe^{-/-}$ group compared with $Ap2^{-/-}Apoe^{-/-}$ controls after 24 hours fasting (data not shown). Insulin tolerance studies showed that insulin sensitivity did not differ between the male $Ap2^{+/+}Apoe^{-/-}$ and $Ap2^{-/-}Apoe^{-/-}$ mice (Fig. 1a). We saw similar results in the females, except that insulin tolerance tests indicated a small improvement in the $Ap2^{+/+}Apoe^{-/-}$ mice compared with $Apoe^{-/-}$ controls (Fig. 1b). These data showed that except for a small change in insulin sensitivity in the females, the steady-state metabolic status of these mice was similar.

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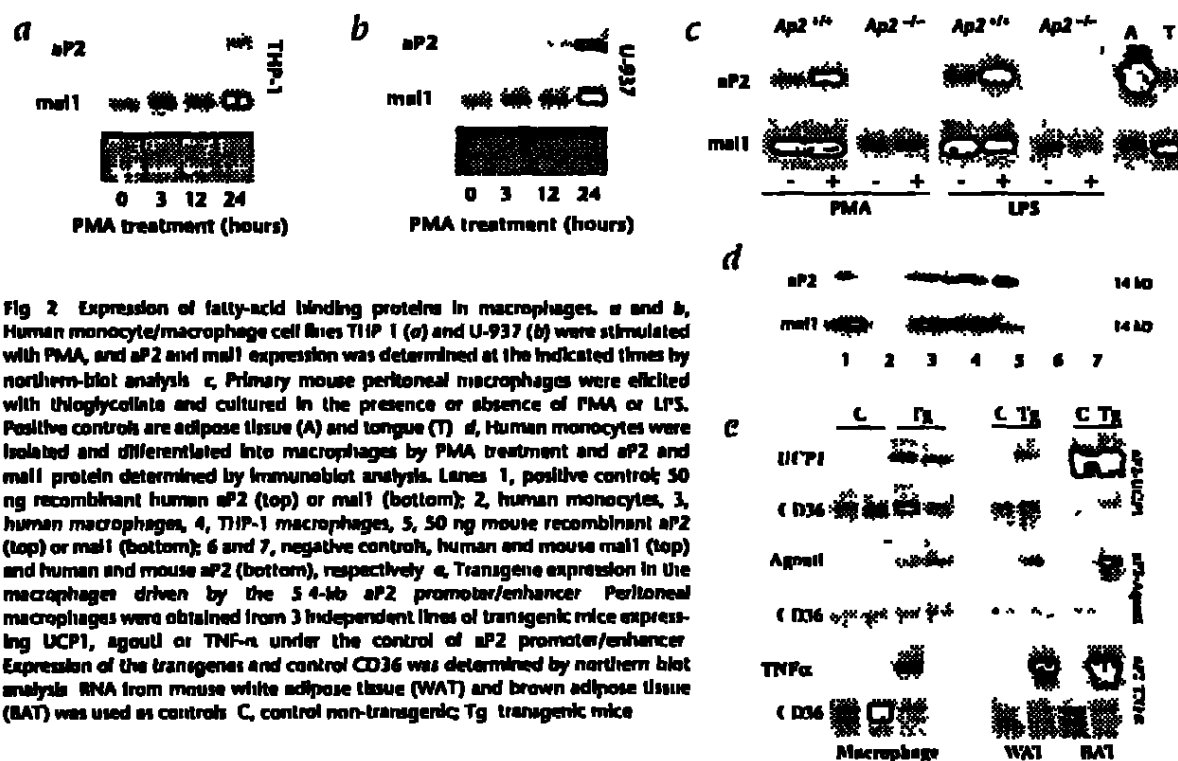


Fig 2 Expression of fatty-acid binding proteins in macrophages. **a** and **b**, Human monocyte/macrophage cell lines THP-1 (**a**) and U-937 (**b**) were stimulated with PMA, and aP2 and mal1 expression was determined at the indicated times by Northern-blot analysis. **c**, Primary mouse peritoneal macrophages were elicited with thioglycollate and cultured in the presence or absence of PMA or LPS. Positive controls are adipose tissue (A) and tongue (T). **d**, Human monocytes were isolated and differentiated into macrophages by PMA treatment and aP2 and mal1 protein determined by immunoblot analysis. Lanes: 1, positive control; 50 ng recombinant human aP2 (top) or mal1 (bottom); 2, human monocytes; 3, human macrophages; 4, THP-1 macrophages; 5, 50 ng mouse recombinant aP2 (top) or mal1 (bottom); 6 and 7, negative controls, human and mouse mal1 (top) and human and mouse aP2 (bottom), respectively. **e**, Transgene expression in the macrophages driven by the 5.4-kb aP2 promoter/enhancer. Peritoneal macrophages were obtained from 3 independent lines of transgenic mice expressing UCP1, agouti or TNF- α under the control of aP2 promoter/enhancer. Expression of the transgenes and control CD36 was determined by Northern blot analysis. RNA from mouse white adipose tissue (WAT) and brown adipose tissue (BAT) was used as controls. C, control non-transgenic; Tg, transgenic mice.

agouti and tumor necrosis factor (TNF)- α under the control of the 5.4-kb aP2 promoter/enhancer. Primary macrophages from all three transgenic lines showed high levels of expression of these transgenes (Fig. 2e). The aP2 promoter/enhancer thus directs expression in macrophages as well as adipocytes.

We next investigated whether aP2 deficiency has functional consequences in macrophage biology that might be relevant to the development of atherosclerosis. We first determined the expression of several inflammatory cytokines, including TNF- α , interleukin (IL)-1 β and IL-6 in Ap2^{-/-} macrophages and wild-type controls. In the resting state, expression of these cytokines was significantly reduced in the aP2-deficient macrophage cell lines compared with Ap2^{+/+} controls (Fig. 3a). Moreover, whereas an increase in TNF- α , IL-1 β , and IL-6 expression was evident in the wild type

cells upon PMA stimulation, we observed no changes in the Ap2^{-/-} cells, demonstrating a reduced inflammatory capacity of Ap2^{-/-} macrophages (Fig. 3a).

To examine the potential function of aP2 in macrophage lipid deposition, cholesterol ester accumulation was assayed in acetylated low-density lipoprotein (Ac-LDL)-loaded macrophages derived from wild-type and Ap2^{-/-} mice. Lipids extracted from cells were assayed using a Nile Red fluorescent-lipid thin-layer chromatography (TLC). Ap2^{-/-} macrophages responded to Ac-LDL loading with 37% less accumulation of cholesterol ester compared with the Ap2^{+/+} controls ($P < 0.001$, Fig. 3b). In the absence of lipid loading, the cholesterol ester levels in the Ap2^{-/-} cells were 29% lower than in Ap2^{+/+} cells ($P < 0.01$). We also evaluated the levels of aP2 and mal1 protein expression in the Ap2^{-/-} and wild-type macrophages. Under the conditions of the lipid accumulation studies, the aP2 protein level in the wild-type line (2 ng/ μ g cell protein) was approximately 22% that of mal1 (9 ng/ μ g). In the Ap2^{-/-} line, aP2 was not detectable and mal1 was 20.9 ng/ μ g. Exposure to Ac-LDL did not affect the expression levels of either

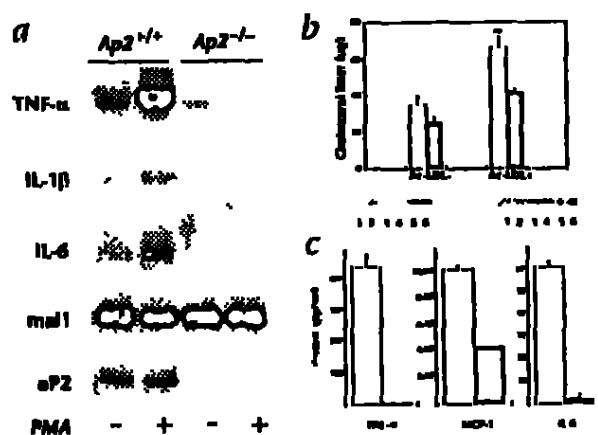


Fig 3 Expression of inflammatory cytokines and cholesterol ester levels in Ap2^{-/-} macrophages. **a**, TNF- α , IL-1 β and IL-6 mRNA levels were determined following PMA treatment. The bottom two blots show mal1 and aP2 mRNA expression. **b** and **c**, Accumulation of cholesterol esters (**b**) and cytokine secretion (**c**) in control (L) and Ap2^{-/-} (R) macrophages. Cholesterol ester levels were determined before and after treatment with Ac-LDL. Cytokine levels were determined by ELISA in the conditioned medium following treatment with Ac-LDL. The graph shows mean $\pm$ s.e. from 10 independent experiments. Immunoblot analysis of aP2 (L) and mal1 (R) protein expression is shown in **b**, lower. Lanes: 1, Ap2^{+/+} without Ac-LDL; 2, Ap2^{+/+} with Ac-LDL; 3, Ap2^{-/-} without Ac-LDL; 4, Ap2^{-/-} with Ac-LDL; 5, 6, 20 and 40 ng of recombinant murine aP2 (left) or Mal1 (right) standards.

Table 1 Serum lipids and glucose in $Ap2^{-/-}$ BMT mice

Group	Serum study	Baseline	4 wk post BMT	8 wk post-BMT	13 wk post BMT
$Ap2^{-/-}Apoe^{-/-} \rightarrow Apoe^{-/-}$ (male), $n = 8$	Cholesterol	350 ± 46	416 ± 80	423 ± 52	448 ± 109
	Triglycerides	147 ± 36	169 ± 54	120 ± 39	137 ± 49
	Glucose		110 ± 18	110 ± 18	127 ± 39
$Ap2^{-/-}Apoe^{-/-} \rightarrow Apoe^{-/-}$ (male), $n = 9$	Cholesterol	331 ± 43	450 ± 37	371 ± 66	395 ± 88
	Triglycerides	135 ± 40	144 ± 50	129 ± 32	135 ± 31
	Glucose		110 ± 9	110 ± 9	135 ± 27
$Ap2^{-/-}Apoe^{-/-} \rightarrow Apoe^{-/-}$ (female), $n = 11$	Cholesterol	282 ± 64	406 ± 67	454 ± 56	300 ± 45
	Triglycerides	63 ± 15	69 ± 19	81 ± 27	98 ± 46
	Glucose		101 ± 31	101 ± 31	94 ± 18
$Ap2^{-/-}Apoe^{-/-} \rightarrow Apoe^{-/-}$ (female), $n = 8$	Cholesterol	265 ± 59	443 ± 85	432 ± 110	283 ± 92
	Triglycerides	53 ± 12	80 ± 16	85 ± 22	118 ± 48
	Glucose		90 ± 42	90 ± 42	107 ± 14

Fasting (4 h) total serum cholesterol, -triglyceride and -glucose levels in male and female $Apoe^{-/-}$ mice before and after BMT with $Ap2^{-/-}Apoe^{-/-}$ or $Ap2^{-/-}Apoe^{-/-}$ marrow. Values (mg/dl) are mean $\pm$ s.d.

1ABP in this model (Fig. 3b, lower panel). These results indicate that genetic elimination of $ap2$ significantly decreased intracellular cholesterol ester accumulation in macrophages despite the presence of substantial amounts of small protein. We also determined cytokine production from these cells under the same experimental conditions. These experiments demonstrated a strong reduction in the capacity of $Ap2^{-/-}$ cells to secrete TNF- α , monocyte chemoattractant protein (MCP)-1, and IL-6 proteins compared with wild-type controls (Fig. 3c).

Bone-marrow transplantation studies

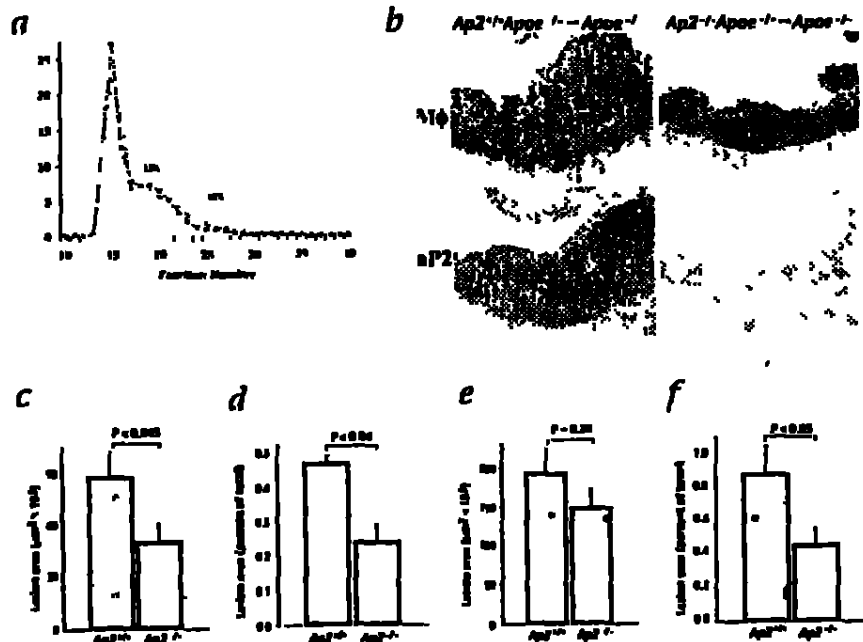
BMT studies in gene-targeted mice have been a powerful tool to examine the contribution of various genes expressed by macrophages to foam-cell formation and atherogenesis^{23,24}. We therefore created $Apoe^{-/-}$ mice chimeric for macrophage $ap2$ ex-

pression using BMT to determine the contribution of macrophage $ap2$ -deficiency in atherogenesis. Ten-week-old male $Apoe^{-/-}$ mice were lethally irradiated (9 Gy) and transplanted with either $Ap2^{-/-}Apoe^{-/-}$ (experimental group, $n = 10$) or $Ap2^{-/-}Apoe^{-/-}$ (control group, $n = 8$) bone marrow. Similarly, 10-week-old female $Apoe^{-/-}$ mice were lethally irradiated and transplanted with either $Ap2^{-/-}Apoe^{-/-}$ (experimental group, $n = 8$) or $Ap2^{-/-}Apoe^{-/-}$ (control group, $n = 11$) bone marrow. The transplanted mice were fed a standard chow diet (< 4.5% fat) for 13 weeks. We observed no significant differences in serum levels of glucose, cholesterol or triglycerides between the experimental and control groups in either experiment at 8 or 13 weeks after transplantation (Table 1). Moreover, the distribution of cholesterol among the serum lipoproteins as determined by size exclusion chromatography did not dif-

fer significantly between the experimental and control groups in either experiment (Fig. 4a). Finally, insulin tolerance tests showed that insulin sensitivity did not differ between the two groups (data not shown). Therefore, the lack of macrophage $ap2$ expression had no impact on fasting serum-glucose and -lipid levels or lipoprotein profiles under these dietary conditions.

Thirteen weeks after transplantation, the extent of atherosclerosis in the proximal aorta of the transplanted $Apoe^{-/-}$ mice was determined. Staining of cross-sections of the proximal aorta with Oil Red O revealed fatty streak lesions, which in the males consisted almost exclusively of macrophage-derived foam cells, as determined by immunocytochemical staining with MOMA-2 (Fig. 4b). Immunocytochemical analysis of serial sections of the proximal aorta by staining with MOMA-2 for mouse macrophages (Fig. 4b, upper panels), or the $ap2$ protein (Fig. 4b, lower panels) revealed

Fig. 4 Lipoprotein distribution, immunocytochemistry and atherosclerosis in $Ap2^{-/-}$ BMT mice. a, Lipoprotein distribution in $Apoe^{-/-}$ mice with transplanted marrow after 13 wk on standard chow diet: $\square$, female $Ap2^{-/-}Apoe^{-/-}$; $\square$, female $Ap2^{-/-}Apoe^{-/-}$; $\square$, male $Ap2^{-/-}Apoe^{-/-}$; $\square$, male $Ap2^{-/-}Apoe^{-/-}$. Data are represented as an average ($n = 3$) percent distribution of total cholesterol for each group. Fractions 14–17 contain VLDL, fractions 18–24 are LDL/LDL₁, and fractions 25–29 contain HDL. Fractions 30–40 are the non-lipoprotein associated proteins. b, Immunocytochemical detection of macrophages and $ap2$ expression in the proximal aorta of $Apoe^{-/-}$ mice transplanted with $Ap2^{-/-}Apoe^{-/-}$ (left) or $Ap2^{-/-}Apoe^{-/-}$ (right) marrow. Macrophages are stained as in Fig. 1c. c–f, Quantification of atherosclerotic lesion area in the proximal and the en face aorta, respectively for male (c and d) and female (e and f) $Apoe^{-/-}$ mice 13 weeks after receiving $Ap2^{-/-}Apoe^{-/-}$ or $Ap2^{-/-}Apoe^{-/-}$ marrow. The atherosclerotic lesions were stained and quantified as noted in Fig. 1. Data are represented as the average mean lesion area for each group.



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that macrophage-derived foam cells in $Ap2^{+/-} \rightarrow Apoe^{-/-}$ mice colocalized with aP2 protein. Macrophages from $Ap2^{+/-} \rightarrow Apoe^{-/-}$ mice did not react with the aP2 antibody. Quantitative analysis of the extent of atherosclerotic lesions in the proximal aorta revealed that mean lesion area in male $Ap2^{+/-} \rightarrow Apoe^{-/-}$ mice was reduced by 43% compared with $Ap2^{+/+} \rightarrow Apoe^{-/-}$ mice ($50,311 \pm 10,600$ and $89,688 \pm 13,940$ μm^2 /section $\pm$ s.e.m., respectively; $P < 0.045$, Fig. 4c). *In vivo* analysis of the extent of atherosclerosis in the entire aorta in $Ap2^{+/-} \rightarrow Apoe^{-/-}$ males revealed a 48% reduction compared with $Ap2^{+/+} \rightarrow Apoe^{-/-}$ males (0.24 ± 0.05 versus $0.47 \pm 0.09\%$, percent $\pm$ s.e.m., $P < 0.04$, Fig. 4d). Thus, male $Apoe^{-/-}$ mice reconstituted with $Ap2^{+/-}$ macrophages are protected from atherosclerosis compared with $Ap2^{+/+} \rightarrow Apoe^{-/-}$ mice. As expected, the extent of atherosclerosis was greater in the female $Apoe^{-/-}$ transplant recipients, and lesions in their proximal aorta were significantly more advanced, showing the presence of smooth muscle cells in the intima. Quantitative analysis of the extent of atherosclerotic lesions in the proximal aorta revealed a trend for a reduction in mean lesion area in female $Ap2^{+/-} \rightarrow Apoe^{-/-}$ mice compared with $Ap2^{+/+} \rightarrow Apoe^{-/-}$ mice ($130,074 \pm 23,347$ and $194,358 \pm 29,798$ μm^2 /section $\pm$ s.e.m., respectively; $P = 0.288$, Fig. 4e). However, *en face* analysis of the extent of atherosclerosis in the entire aorta in $Ap2^{+/-} \rightarrow Apoe^{-/-}$ females revealed a significant 48% reduction in mean lesion area compared with $Ap2^{+/+} \rightarrow Apoe^{-/-}$ mice (0.46 ± 0.05 versus 0.89 ± 0.16 , percent $\pm$ s.e.m., $P < 0.04$, Fig. 4f). These transplantation studies demonstrate a role for macrophage aP2 expression in promoting foam-cell formation and atherogenesis *in vivo*.

Discussion

Previous studies have demonstrated that deficiency of aP2, a downstream target of PPAR- γ , predominantly affects adipocytes and contributes to improved systemic glucose and lipid metabolism in the setting of dietary or genetic obesity²². Here, we demonstrate that aP2 is also strongly expressed in macrophages and modulates their biological responses. Total and macrophage-specific aP2 deficiencies led to a striking protection from the development of atherosclerosis in the $Apoe^{-/-}$ model. Based on these data, a potential mechanism underlying the metabolic syndrome emerges from coordinated modulation of the metabolic status of adipocytes and the metabolic or inflammatory status of macrophages through pathways common to both cell types.

The gene encoding aP2 is expressed at high levels in mouse and human macrophages upon activation *in vitro* and in macrophages associated with atherosclerotic lesions *in vivo*, raising the possibility that it might impact the macrophage inflammatory response and foam-cell formation. As in adipocytes, production of TNF- α is greatly reduced in $Ap2^{-/-}$ macrophages compared with wild type controls²². Other inflammatory cytokines such as IL-1 β and IL-6 are also suppressed in $Ap2^{-/-}$ macrophages. Moreover, $Ap2^{+/-}$ macrophages display significantly decreased intracellular cholesterol ester accumulation *in vitro* and secrete highly reduced quantities of TNF- α , MCP-1 and IL-6 upon exposure to modified lipoprotein. These results indicate that aP2 has a significant role in two important aspects of macrophage biology that are highly relevant to the pathogenesis of atherosclerosis: m1a1, a close relative of aP2, presents an expression pattern similar to aP2. However, whereas the expression of m1a1 mRNA in $Ap2^{-/-}$ adipocytes is greatly enhanced, compensatory regulation in $Ap2^{-/-}$ macrophages is mild. Hence, it will be important to determine the impact of m1a1 and combined aP2/m1a1 deficiency in metabolic regulation and atherosclerosis. Finally, macrophage aP2 expression is controlled with the same promoter/enhancer

elements that confer expression in adipocytes; this might have important implications in the assessment of many transgenic lines generated with the 5.4-kb aP2 promoter/enhancer.

Both macrophages and adipocytes are sites for active lipid metabolism and signaling. Therefore, it is not surprising that FABP4 have a strong impact on the biology of these cells. This is likely to involve many aspects of lipid metabolism in both cell types including transport of lipids and shuttling them to specific metabolic or signaling pathways. Recent studies with other isoforms have indicated potential regulation of nuclear hormone receptor activity in the liver through direct transportation of lipid and xenobiotic ligands²⁴. Regulation of macrophage ACAT activity^{25,26} or the availability of FA for esterification with cholesterol might also be altered by FABP4. Alternatively, FABP4 could impact the rates of transport through quantitative or functional alterations in fatty-acid transporters or scavenger receptors. Further studies will be required to address these possibilities.

A critical question is whether the function of aP2 in the macrophage is sufficient to modulate development of atherosclerosis in a way that is distinguishable from the metabolic consequences of aP2 deficiency. We have taken several steps to address this question. First, we studied the effect of aP2 deficiency on the development of atherosclerosis in $Apoe^{-/-}$ mice on normal chow diet. On this diet, $Apoe^{-/-}$ mice exhibit severe hypercholesterolemia and spontaneous atherosclerotic lesions in the absence of significant changes in body weight or serum levels of glucose or insulin. Under these conditions, male $Ap2^{+/-}Apoe^{-/-}$ mice, despite severe hypercholesterolemia, developed 66% less atherosclerosis in cross-sections of the proximal aorta and 86% smaller lesions by *en face* analysis of the extent of lesions in the entire aorta compared with control $Ap2^{+/+}Apoe^{-/-}$ mice. These reductions in the extent of atherosclerosis occurred in the absence of significant changes in serum lipid, glucose or insulin levels, supporting the hypothesis that aP2 expression contributes to atherosclerosis independent of its established role in glucose and lipid metabolism.

Second, we generated macrophage-restricted aP2 deficiency through RM1 experiments to examine the hypothesis that macrophage aP2 expression promotes atherogenesis independent of its expression by adipocytes. Male $Apoe^{-/-}$ mice reconstituted with $Ap2^{-/-}$ macrophages had a 43% and 49% reduction in lesion area compared with those reconstituted with $Ap2^{+/+}$ cells, as assessed by both lesion size in cross sections of the proximal aorta and in the entire aorta *en face*, respectively. As expected, the extent of atherosclerosis was greater in the proximal aortas of female $Apoe^{-/-}$ transplanted mice than in the males, and the reduction in vascular lesions in female $Ap2^{+/-}Apoe^{-/-} \rightarrow Apoe^{-/-}$ mice at this site was not statistically significant. Although this observation indicates a potential gender difference regarding the effect of aP2 expression in atherosclerosis, we think that it most likely reflects the fact that the lesions in the proximal aorta of the females were more advanced. These include intermediate lesions with smooth muscle cell involvement, indicating that the contribution of macrophage aP2 expression might be lessened in the setting of more advanced lesions. In the whole aorta, a significant 48% reduction in mean lesion area was evident in female $Ap2^{+/-}Apoe^{-/-} \rightarrow Apoe^{-/-}$ mice compared with female $Ap2^{+/+}Apoe^{-/-} \rightarrow Apoe^{-/-}$ mice. This may be explained by the fact that mice develop atherosclerotic lesions first in the proximal aorta and the lesions progress distally, resulting in more advanced lesions in the proximal than the distal aorta²⁷. Moreover, in response to 14 weeks on the Western-type diet, female $Apoe^{-/-}Ap2^{+/-}$ mice show reduced atherosclerosis in the proximal aorta compared with $Apoe^{-/-}Ap2^{+/+}$ con-

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trois, indicating that the effects of *ap2* on atherosclerosis are not gender specific. These results demonstrate a significant role for macrophage *ap2* in atherogenesis independent of its expression in adipocytes and its role in glucose and lipid metabolism.

Taken together with the previous observations regarding the impact of *ap2* on glucose and lipid metabolism, our data support an important role for *ap2* in understanding the molecular basis of metabolic syndrome via its coordinated action on both metabolic and inflammatory responses. These findings should provide further insights into the molecular mechanisms leading to these diseases and generate opportunities for the development of a novel class of therapeutic modalities to treat them.

Methods

Animal procedures. Mice homozygous for inactivation of *Ap2* were backcrossed 12 generations into the C57BL/6 background²⁴. These *Ap2*^{-/-} mice were intercrossed with the *ApoE*-deficient mice also in the C57BL/6 background to generate mice heterozygous at both loci. These *Ap2*^{+/-}*ApoE*^{+/-} mice were then intercrossed to produce *Ap2*^{-/-}*ApoE*^{-/-} mice along with *Ap2*^{+/-}*ApoE*^{-/-} littermate controls. At 4 weeks, littermates were divided into an experimental group of *Ap2*^{-/-}*ApoE*^{-/-} and a control group of *Ap2*^{+/-}*ApoE*^{-/-} mice. For the BMT study, donor mice were derived from the same colony of *Ap2*^{+/-}*ApoE*^{-/-} and *Ap2*^{-/-}*ApoE*^{-/-} mice described above. Recipient mice were derived from an established colony at our mouse facility of *ApoE*-deficient mice on the C57BL/6 background²⁴. Mice were fed standard chow with 4.5% fat (PMI Feeds, St. Louis, Missouri) or Western diet (diet #D88137, Harlan Teklad, Madison, Wisconsin). UCP1 and agouti transgenic mice were provided by L. Kozak. Transgenic mice expressing a membrane-bound form of TNF- α driven by the *ap2* promoter/enhancer in the TNF- α -deficient background were generated in one of our laboratories (Harvard). Animal care and experimental procedures were performed under approval from the Animal Care Committee of Vanderbilt and Harvard Universities.

Bone-marrow transplantation. 1 week before and 2 weeks following BMT, 100 mg/L neomycin and 10 mg/L polymyxin B sulfate (Sigma) were added to the acidified water. Bone marrow was collected from donor mice by flushing femurs with RPMI 1640 media (GIBCO, Grand Island, New York) containing 2% FBS and 5 U/ml heparin (Sigma). Recipient mice were lethally irradiated (9 Gy) by a cesium gamma source. 4 hours later, 5–10 $\times 10^6$ bone-marrow cells in 0.3 ml were transplanted by tail-vein injection as described²⁵.

Serum measurements and insulin tolerance tests. Mice were fasted for either 4 h during day or 24 h overnight and blood samples were collected by retro-orbital venous plexus puncture under isoflurane anesthesia (Isovet, Schering-Plough, Union, New Jersey). Serum was separated by centrifugation and 1 mM phenylmethylsulfonyl fluoride was added (Sigma). The serum total cholesterol and triglycerides were determined using Sigma kit #352 and kit #339 adapted for microtiter plate assay as described²⁶. Blood glucose concentrations were determined on 5 μ l whole blood by using gluco-analyser blood glucose strips (Midasense, Bedford, Massachusetts). Steady-state insulin concentrations were determined with a commercially available radioimmunoassay (Linco, St. Charles, Missouri). Lipoprotein assays were performed as described²⁷. Insulin tolerance tests (0.5 IU/kg) were performed on conscious mice following a 6-h fast as described^{28,29}.

Immunocytochemistry and quantification of arterial lesions. To detect macrophages and the *ap2* protein in arterial lesions, 5- μ m serial cryosections of the proximal aorta were incubated with either a polyclonal rabbit antiserum against mouse *ap2* (gift of D. Bernlohr) or rat antibody against mouse macrophage³⁰, MOMA-2 (Accurate, Westbury, New York) as described³¹. For quantification of arterial lesions, aortas were pinned out in an en face preparation as described^{32,33}. 10- μ m thick cryosections of the proximal aorta were stained with Oil Red O and counterstained with hematoxylin, as described^{34,35}. Quantitative analysis of lipid-stained lesions was performed using an Imaging System KS 300 (Release 2.0; Kontron Elektronik GmbH, Eden Prairie, Minnesota). Color threshold was used to define the Oil-Red-O-stained lesion area that was measured as squared microns per mouse.

Macrophage studies. PBMCs were prepared from healthy donors as described³⁶ and were incubated overnight in RPMI medium + 5% FBS to spontaneously differentiate into macrophages. THP-1 (ATCC #TIB 202) and U 937 (ATCC #CRL-1593.2) human monocytic leukemia cell lines were grown in RPMI with 5% FBS and differentiated by 25 or 100 nM PMA into macrophages. Immortalized *Ap2*^{+/+} and *Ap2*^{-/-} murine macrophage cell lines were generated in our laboratory by a modification of a described procedure³⁷. Primary macrophages are isolated from the peritoneal cavity and were incubated with the indicated reagents for 24 hours. *Ap2*^{+/+} and *Ap2*^{-/-} mouse macrophage cell lines were grown to near confluence in 12-well plates in RPMI 1640 with 5% FBS. Supernatants were collected after 72 h of treatment with 50 μ g/ml Ac-LDL (Biomedical Technologies, Stoughton, Massachusetts) and cytokine levels were determined by commercially available ELISA systems (TNF- α from R&D, Minneapolis, Minnesota, IL-6 and MCP-1 from PharMingen, San Diego, California). Macrophage cell lines were cultured and lipids were extracted from the cells for TLC analysis as described³⁸. Immunoblots were conducted with rabbit antibodies against human recombinant *ap2* and *mal1* as described³⁹.

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REVISIONES

Tratamiento combinado de la diabetes tipo 2

Combined treatment of type 2 diabetes

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INTRODUCCIÓN

La diabetes mellitus tipo 2 (DM tipo 2) afecta a más del 10% de la población española mayor de 35 años de edad¹ y su prevalencia no cesa de aumentar en los países desarrollados. Los pacientes diabéticos tienen un incremento en su morbilidad cardiovascular de al menos 3 veces lo que disminuye su expectativa de vida y presentan con gran frecuencia complicaciones microvasculares (retinopatía, nefropatía y neuropatía)², todo lo cual acarrea un alto coste social y sanitario al afectar de una forma muy importante a su calidad de vida.

Se ha demostrado que la mejora del control glucémico en estos pacientes es capaz de reducir la incidencia y la progresión de las complicaciones microvasculares³. En la prevención de las complicaciones el control de la glucemia debe formar parte de un tratamiento integral de todos los factores de riesgo cardiovascular, los cuales suelen agregarse en este grupo de enfermos, como son la hipertensión arterial, la hiperlipemia, la obesidad o el tabaquismo⁴.

Así pues en el momento actual, el tratamiento de la diabetes mellitus tipo 2 va dirigido no sólo a evitar la clínica de la hiperglucemia y las descompensaciones metabólicas agudas, sino a evitar la aparición o enlentecer la progresión de las complicaciones crónicas, tanto macro como microvasculares, consiguiendo de esta forma una mejor calidad de vida y una disminución de la mortalidad en este grupo cada vez mas amplio de pacientes⁵.

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TERAPIA COMBINADA

Como ya hemos visto en la DM tipo 2 concurren en mayor o menor medida distintas alteraciones fisiopatológicas y en la actualidad disponemos de fármacos con distintos mecanismos de acción que intentan corregir cada una de ellas

Por otro lado el estudio UKPDS⁴ ha demostrado claramente que la DM tipo 2 es una enfermedad progresiva y que la terapia de combinación con agentes de actividad diferente puede ser necesaria tempranamente ya que a los 3 años de evolución de su enfermedad, sólo el 50% de los diabéticos tipo 2 tenían un control aceptable y la mayoría de los pacientes necesitarán insulina con el tiempo

El tratamiento combinado hace que sean necesarias menores dosis de los fármacos y como consecuencia de ello los efectos adversos disminuirán.

Existen múltiples posibilidades de combinaciones de tratamientos orales y en general los estudios clínicos han demostrado el efecto aditivo hipoglucemiante, que es similar al que obtienen en monoterapia cuando se compara con placebo

Con este tipo de tratamiento conseguiremos mejorar la glucemia y retrasar la instauración de la insulinoterapia, pero hoy por hoy no ha demostrado sus beneficios frente a órganos diana.

Cuando no se consigue un buen control con tratamiento combinado oral se debe pasar al tratamiento insulínico combinado con el oral o bien en monoterapia.

Secretagogos de insulina + metformina

Aunque no ampliamente utilizada hasta hace poco tiempo, es una asociación razonable puesto que los secretagogos mitigarán la deficiente secreción de insulina y la metformina disminuirá la excesiva producción hepática de glucosa.

La adición de metformina a pacientes tratados con sulfonilureas⁵³⁻⁵⁵, repaglinida⁵⁶, o nateglinida⁵⁷, bajan la HbA1c de un 1,5 a un 2,2% sin aumentar significativamente los efectos secundarios

También se ha observado una mejoría del perfil lipídico con disminución del colesterol total, colesterol LDL y triglicéridos y aumento del colesterol HDL cuando se asocia la metformina⁵⁸

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La mejoría del control es independiente del fármaco con el que se haya comenzado el tratamiento, siendo similar al añadir metformina al secretagogo o secretagogo a la metformina⁵⁹⁻⁶⁰

En el estudio UKPDS el tratamiento combinado de metformina y glibenclámda resultó en un paradójico incremento de la mortalidad general, hallazgo sorprendente y discutido por los propios autores⁴⁷. Un segundo estudio observacional obtuvo resultados similares con esa misma asociación⁶¹, si bien no queda claro si el exceso de mortalidad se debía a la pauta terapéutica o al hecho de que se aplicara a pacientes peor controlados, por lo que este asunto está aún lejos de ser resuelto.

En la actualidad esta asociación es considerada segura y de primera elección cuando fracasa la monoterapia con cualquiera de los fármacos⁶⁻⁹, habiéndose comercializado incluso en los últimos tiempos una asociación preestablecida en un mismo comprimido.

El tratamiento adicional con metformina en un paciente tratado previamente con secretagogos de insulina no induce aumento del peso corporal y las hipoglucemias pueden aparecer al comienzo del tratamiento tras mejorar la resistencia a la insulina, por lo que se requiere un control glucémico frecuente y deben respetarse escrupulosamente las contraindicaciones conocidas.

En algunos casos se podrá sustituir el tratamiento con insulina por esta asociación, sobre todo en pacientes obesos de no muy larga evolución⁶², siempre que haya posibilidades de consulta inmediata en el caso de producirse una descompensación importante y se programe una consulta a corto plazo.

Esta combinación es una opción en aquellos diabéticos tipo 2 con o sin sobrepeso en tratamiento con secretagogos de insulina o metformina en monoterapia, con deficiente control y en el que no existen contraindicaciones. Ha demostrado que puede retrasar la necesidad de insulinización en unos 8 años de media⁶³.

Sulfonilureas + inhibidores de las α -glucosidasas

Tras asociar a una sulfonilurea un inhibidor de las α -glucosidasas (acarbose o miglitol) se produce un efecto sinérgico y una reducción de las glucemias postprandiales por el retraso en la absorción de carbohidratos por efecto de estos últimos, consiguiéndose una disminución de la HbA1c del 0,5 al 1% sin cambios en la insulinemia ni en la incidencia de hipoglucemias⁶⁴⁻⁶⁵.

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No debe olvidarse que las hipoglucemias siempre que se tomen inhibidores de las α -glucosidasas deben corregirse con dextrosa pura.

La mayor limitación de esta asociación es la mala tolerancia por los frecuentes efectos secundarios gastrointestinales de los inhibidores de las α -glucosidasas

Esta combinación estará indicada en pacientes en tratamiento con sulfonilureas e insuficiente control glucémico debido a glucemias posprandiales elevadas, pero es inútil en pacientes en tratamiento con dosis máximas de sulfonilureas y glucemias basales persistentemente elevadas

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control glucémico debido a glucemias posprandiales elevadas, pero es inútil en pacientes en tratamiento con dosis máximas de sulfonilureas y glucemias basales persistentemente elevadas.

Metformina + inhibidores de las α -glucosidasas

Los resultados de esta asociación, consecuencia de la suma del efecto inhibitor de la gluconeogénesis de la metformina y la menor absorción de carbohidratos de los inhibidores de las α -glucosidasas, son peores que los de la asociación de secretagogos con metformina y se consigue únicamente una reducción de la HbA1c del 0,7%⁶⁵ al 0,8%⁶⁶

Se debe tener en cuenta el riesgo de que se potencien sus efectos secundarios gastrointestinales que hacen que un porcentaje importante de pacientes abandone el tratamiento

Ninguno de los dos grupos de fármacos tiene efectos negativos en el peso ni producen por sí mismos hipoglucemias por lo que no existe riesgo de esta complicación

Esta asociación sería una opción para los pacientes que estando en tratamiento con metformina mantienen fundamentalmente glucemias postprandiales altas y un control inadecuado

Sulfonilureas + tiazolidíndionas

La adición de troglitazona a pacientes tratados con glibenclamida demostró una reducción de la HbA1c dependiente de la dosis entre 0,7 y 1,7 puntos⁶⁷ y efectos similares han demostrado en asociación con sulfonilureas tanto la rosiglitazona⁶⁸ como la pioglitazona⁶⁹

Estas combinaciones mejoran la resistencia a la insulina como atestiguan una reducción significativa de la hiperinsulinemia en pacientes insulinoresistentes, pero debe tenerse en cuenta que producen aumento de peso y del colesterol LDL, aunque mejoran el HDL-colesterol y los triglicéridos

En España, la rosiglitazona está aprobada para esta combinación de tratamiento sólo en asistencia especializada.

Esta asociación sería una opción para paciente en tratamiento con sulfonilureas, mal control metabólico y sobrepeso, obesidad o resistencia a la insulina y contraindicación o intolerancia de la metformina

Metformina + tiazolidíndionas

Esta combinación se ha demostrado eficaz en recientes ensayos clínicos disminuyendo la HbA1c

una media de 1-1,2 puntos en grupos de pacientes con BMI superior a 2. En todos los casos se produce un aumento de peso y se observa también un aumento del LDL-Colesterol, sin que aumenten los efectos secundarios gastrointestinales y sin presentar toxicidad hepática^{70,71}

La indicación admitida en Europa para la pioglitazona por el momento es únicamente en esta asociación.

Triple terapia oral secretagogo + metformina (o glitazonas) + inhibidor de las α -glucosidasas

La asociación de un secretagogo, metformina o glitazonas y un inhibidor de las α -glucosidasas es una opción posible que puede evitar la insulinización en algunos pacientes con problemas para la inyección, pero la experiencia es escasa y el beneficio esperado sólo marginal⁷²

El Consenso Europeo admite la triple terapia oral cuando el control no es adecuado con dos fármacos⁸

En la tabla 5 se presentan resumidas las ventajas e inconvenientes de las diferentes pautas de tratamiento combinado oral

Sulfonilureas + insulina

<http://www.cfinavara.es/salud/analisis/textos/vol25/n2/revista.html>

Tabla 5 Características de los tratamientos combinados orales.

	Secretagogos + metformina	Secretagogos + inhibidores de las α -glucosidasas	Metformina + inhibidores de las α -glucosidasas	Sulfonilureas + tiazolidinedionas	Metformina + tiazolidinedionas
Mejoría de la glucemia	Glucemia basal	Glucemia postprandrial	Glucemia postprandrial	Glucemia basal	Glucemia basal
Reducción de la HbA1c	~ 2 %	~ 0,5 %	~ 0,5 %	~ 1,2 %	~ 1,4 %
Peso	Aumento al añadir el secretagogo	Aumento al añadir el secretagogo	Mantenimiento	Aumento	Mantenimiento
Efectos secundarios	Gastrointestinales (~ 25 %) Acidosis láctica (Rara)	Flatulencia (~ 40 %)	Flatulencia (~ 30 %) Diarreas (~ 30 %) Acidosis láctica (Rara)	Ledemas Insuficiencia cardíaca Posible elevación de transaminasas	Edemas IC posible hepatotoxicidad Alt. gastrointestinales Acidosis láctica (Rara)

22/10/2002

Durante muchos años y debido a que su mecanismo de acción es similar no se han llevado a cabo estudios clínicos de entidad significativa del tratamiento combinado de sulfonilureas con insulina, pero en la última década se han realizado varios metaanálisis de los trabajos previos⁷³⁻⁷⁵ y nuevos estudios⁷⁶⁻⁷⁷ que han mostrado sin lugar a dudas que la asociación de sulfonilureas a pacientes con DM tipo 2 tratados previamente con insulina disminuye tanto la glucemia como la HbA1c, permitiendo una reducción en la dosis necesaria de insulina

Se ha planteado cuál sería la mejor pauta de administración de insulina en combinación con sulfonilureas y diversos ensayos han demostrado que la administración nocturna consigue un control metabólico similar provocando menor hipoglucemia y menor aumento de peso sin aumentar el riesgo de hipoglucemias⁷⁸

El efecto de la insulina durante la noche inhibe la producción hepática de glucosa, yugula la hiperglucemia de primera hora de la mañana y de esta forma mejora la glucemia basal, condicionante fundamental de la hiperglucemia post-prandial durante el resto del día⁷⁹

En la práctica clínica se continúa con las sulfonilureas a la dosis previa y se añade una dosis única de insulina NPH antes de la cena o antes de ir a la cama⁷⁷ y en ocasiones se puede valorar la inyección de una mezcla de insulina NPH y rápida antes de la cena⁸⁰. La dosis de comienzo debe ser baja, de 5 a 10 unidades en pacientes en normopeso y de 10 a 15 U en paciente obesos, ajustándose posteriormente en función de la glucemia basal y la clínica a intervalos semanales. Si las glucemias diurnas bajan excesivamente se debe reducir la dosis de sulfonilureas⁸¹

Por supuesto, la instauración del tratamiento con insulina exige realizar un programa mínimo de educación en diabetes, incluyendo la autoinyección, el autocontrol de glucemia en sangre y el manejo de las hipoglucemias, que se hace ambulatoriamente de forma sencilla. La aceptación por parte del paciente y la adherencia al tratamiento es muy buena.

Metformina + insulina

La metformina por su mecanismo de acción es un buen fármaco para ser combinado con la insulina exógena en el tratamiento de pacientes diabéticos tipo 2.

Diversos estudios han demostrado los beneficios de esta asociación, ya que consigue en pacientes con mal control previo en tratamiento con sulfonilureas⁸² o con insulina⁸³⁻⁸⁴, una importante mejoría de la HbA1c que disminuye hasta un 2,4% con un menor requerimiento de insulina y todo ello produciendo un mínimo aumento de peso. Por otra parte la adición de metformina mejora el perfil lipídico.

Esta asociación es una alternativa en diabéticos tipo 2 tratados con insulina cuyo control glucémico no es adecuado, especialmente en aquellos pacientes obesos o con resistencia a la insulina.

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Se recomienda comenzar con dosis bajas de medio comprimido cada 12 horas para evitar los efectos secundarios gastrointestinales, aumentando la dosis a las 2-4 semanas en caso necesario y posteriormente debe valorarse la posibilidad de reducir la dosis de insulina.

Inhibidores de las α -glucosidasas + insulina

En asociación con insulina tanto la acarbosa⁸⁵ como el miglitol⁸⁶ disminuyen la HbA1c si las comparamos con placebo, pero esta reducción es modesta, de un 0,4 a un 0,7%. Su principal inconveniente sigue siendo los efectos secundarios gastrointestinales que limitan su utilización.

Podría considerarse esta combinación en aquellos pacientes previamente tratados con insulina y con glucemias postprandiales elevadas.

Tiazolidíndionas + insulina

La asociación de glitazonas con insulina exógena permite atacar los dos defectos fundamentales de la DM tipo 2, la deficiencia de insulina y la menor sensibilidad a esta hormona.

Los estudios iniciales con troglitazona⁸⁷ demostraron que su adición a la insulina producía una mejoría de la HbA1c dependiente de la dosis de hasta un 1,4%, permitiendo además la reducción de la dosis de insulina necesaria. Incluso un estudio de comparación directa⁸⁸ ha observado un mayor efecto ahorrador de la insulina de la combinación con troglitazona que la combinación con metformina.

Ensayos clínicos con rosiglitazona⁸⁹ han demostrado también su efectividad en la mejoría del control glucémico sin efectos secundarios graves. Sin embargo, en el momento actual la indicación de asociación de estos fármacos con insulina no ha sido aún aprobada por la Agencia Europea del Medicamento.

CONCLUSIONES

El tratamiento de los pacientes con DM tipo 2 se basa en el control glucémico y del resto de los factores de riesgo cardiovascular, para evitar la aparición de las complicaciones crónicas o en el peor de los casos enlentecer su desarrollo, de tal forma que disminuya la mortalidad y mejore de una forma sustancial la calidad de vida.

La variabilidad de la fisiopatología subyacente en la DM tipo 2 hace de ella una entidad sindrómica más que una entidad nosológica. Como ha enseñado el UKPDS conseguir un buen control de la DM tipo 2 es muy difícil debido a la naturaleza progresiva de la enfermedad, sin embargo una mejora a largo plazo del control glucémico merece la pena. Los resultados en monoterapia subrayan un papel preponderante para la metformina, la única que ha demostrado reducir la mortalidad y en menor grado para los glibenclamida y el tratamiento insulínico.

Todos los consensos y recomendaciones de sociedades científicas abogan por un tratamiento por pasos similar al propuesto en la figura 2, comenzando por la dieta y los cambios en el estilo de vida y pasando en función del objetivo de control de la hemoglobina glicosilada a la monoterapia oral. Si con monoterapia no se consiguen los objetivos de control, lo cual ocurre en un porcentaje importante de pacientes a los pocos años, se pasará al tratamiento combinado oral, para lo cual se dispone en la actualidad de varios grupos de fármacos, algunos de ellos de reciente introducción, con distintos mecanismos de acción. Se pueden utilizar diversas combinaciones de estos fármacos orales entre sí o bien con insulina, cuando ésta sea considerada necesaria habiéndose demostrado en estos últimos años su eficacia en ensayos clínicos randomizados prospectivos a medio plazo.

Las posibles combinaciones son numerosas y el tipo de asociación necesario debe ser elegido en función de los objetivos de control individuales, el mecanismo fisiopatológico presuntamente implicado en cada caso, la eficacia, coste y efectos secundarios de cada fármaco así como las características de cada paciente.

El tratamiento combinado es pues una alternativa válida en el control de la DM tipo 2, aunque quedan por demostrar su eficacia a largo plazo sobre la morbimortalidad en estos pacientes.

No debemos olvidar que la dieta y el ejercicio siguen siendo fundamentales para conseguir los objetivos terapéuticos y que es imprescindible la participación activa del enfermo en su autocuidado, siempre en armonía con sus cuidadores, médico y enfermera educadora. Además es imprescindible un estricto control y un tratamiento agresivo de la hiperlipidemia y de la hipertensión arterial conocida su rentabilidad en términos de reducción de complicaciones macro y microvasculares así como de las tasas de morbimortalidad en general.

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Tables for
New Oral Antihyperglycaemic Agents Expand Armamentarium in the Battle Against Type 2 Diabetes Mellitus

[Drug Ther Perspect 12(7):8-10, 1998 © 1998 Adis International Limited]

Table 1. Effects of combining different antihyperglycaemic drugs in patients with type 2 diabetes mellitus^[1]

Drug combination	Sulphonylurea	Metformin	Acarbose	Troglitazone
Insulin	Significant endogenous secretion of insulin prerequisite for this combination	Reduces insulin requirement in obese patients, improves glycaemic control and/or corrects associated metabolic abnormalities	Reduces blood glucose variations	Favourable effects on glycaemic control and insulin requirement in patients who are poorly controlled despite multiple insulin injections >30 U/day
Sulphonylurea	—	Commonly used combination. Additive antihyperglycaemic effect without increasing the adverse effects of either drug	As effective as sulphonylurea-metformin combination	Useful combination in patients not well controlled by sulphonylureas alone
Acarbose	As effective as sulphonylurea-metformin combination	Shown to be effective. Bioavailability of metformin could be reduced by around 35%	—	Not yet evaluated
Troglitazone	Useful combination in patients not well controlled by sulphonylureas alone	Well tolerated. Additive effects on fasting plasma glucose and HbA _{1c} levels, and insulin-mediated glucose disposal in obese patients	Not yet evaluated	—

Abbreviation HbA_{1c} = glycosylated haemoglobin

Table 2. Selection of an oral antihyperglycaemic drug in patients with type 2 diabetes mellitus according to glycaemic control^[1,2]

Fasting plasma glucose level [mmol/L (mg/dl)]	Other patient characteristics	Preferred drug therapy
<7.8 (140)	Mainly fasting hyperglycaemia	Metformin
	Prominent postprandial hyperglycaemia	Acarbose
	Severe insulin resistance	Troglitazone

7.8-11.1 (140-200)	Mildly to moderately overweight	Low doses of a sulphonylurea
	Obese	Metformin
>11.1 (200)	Overweight to moderately obese	Sulphonylurea
	Very obese	Metformin

955
989

Table 3. Differential features: Comparison of various features of some oral antihyperglycaemic drugs
[1-4]

Feature	Glibenclamide (glyburide)	Metformin	Acarbose†	Troglitazone†
Class	Sulphonylurea	Biguanide	alpha-Glucosidase inhibitor	Thiazolidinedione
Primary mode of action	Stimulate insulin release	Decrease hepatic glucose output, stimulate peripheral glucose uptake, increase intestinal glucose use	Inhibit digestion of carbohydrates	Enhance insulin action
Principal adverse effects				
hypoglycaemia	Yes	No	No	No
bodyweight gain	Yes	No	No	No
lactic acidosis	No	Yes	No	No
GI symptoms	Yes	Yes	Yes	Yes
Treatment recommendations for specific patient subgroups				
obese patients	x	✓	✓	✓
elderly patients	x ^a	! ^b	✓	-
patients with renal impairment	!	x	✓	✓
patients with hepatic impairment	!	x	x	!
pregnant women	x ^d	x	x	x
breastfeeding women	x	x	x	x

†Acarbose is not available in Denmark. Troglitazone is not available in Australia, Denmark, France, Sweden and The Netherlands, since the withdrawal of troglitazone from the UK market in late 1997, Glaxo Wellcome has withdrawn the applications for regulatory approval to market the drug across the European Union until the risk/benefit ratio from evaluations conducted in the US and Japan are known

^aGlibenclamide is not recommended in elderly patients. Other sulphonylureas may be used with caution except for chlorpropamide and a long-acting formulation of glipizide (Ozida®) which is available in France; this product is contraindicated in patients >=65 years of age

^bMay be used in elderly patients with normal renal and hepatic function who are free of significant cardiovascular or pulmonary disease.

^cContraindicated in severe renal impairment.

^dInsulin therapy is usually substituted. However, if glibenclamide (or other sulphonylureas) is used it should be stopped >=2 days before delivery

Abbreviation and symbols GI = gastrointestinal, x = not recommended, 3 = recommended, ! = use with caution,
 - = insufficient data to make a recommendation

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 968 956

