Laboratory Investigation (2010) 90, 963–965

CORRIGENDUM

Knockdown of FABP5 mRNA decreases cellular cholesterol levels and results in decreased apoB100 secretion and triglyceride accumulation in ARPE-19 cells

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Laboratory Investigation (2010) **90,** 963–965; doi:10.1038/labinvest.2010.87

Correction to: *Laboratory Investigation* (2010) **90,** 906–914 (this issue); doi:10.1038/labinvest.2009.33

The first author submitted the article, which was published on 11 May 2009 without the knowledge of two of the authors. Below are the amendments to the original article that reflect the interpretations of all the authors.

INTRODUCTION

The third paragraph should read:

In this study, we found that knockdown of *FABP5* gene expression resulted in (1) decreased cellular cholesterol and cholesterol ester, (2) increased cellular FFAs and triglycerides, and (3) decreased secretion of apoB100 protein in ARPE-19 cells. These observations indicate that *FABP5* is critical in lipid metabolism in ARPE-19 cells, suggesting that FABP5 downregulation in the RPE/choroid complex *in vivo*[1] might contribute to age-related changes and early AMD.

RESULTS

These sections are amended as follows:

Decreased BODIPY-FA Uptake

Liao *et al*[2] reported that BODIPY-FA uptake is closely correlated with the uptake and metabolism of radiolabeled FAs. To determine the effect of *FABP5* gene knockdown on the influx and metabolism of FAs into siRNA-treated cells, we performed BODIPY-FA uptake assays and found that the mean V values for BODIPY-FA uptake at 24, 48, and 72 h after FABP siRNA treatment were decreased by 5, 8 ($P<0.05$), and 6 % ($P<0.05$), respectively (Figure 3). These results suggest that BODIPY-FA uptake is mediated, in part, by *FABP5*. However, the biological effect appears to minimal.

Increased Cellular Lipid Deposits

We next investigated the effect of *FABP5* knockdown on neutral lipid accumulation in siRNA-treated cells by performing the Nile Red binding assay. Cells treated with FABP5 siRNA developed large Nile Red-stained lipid deposits compared with NTC control. To amplify the effect of the knockdown of FABP5 on neutral lipid deposition, we challenged siRNA-treated cells with oleic acid, which increases the synthesis of triglycerides.[3] Figure 4 shows a marked increase in the size of lipid deposits in FABP5 siRNA-treated cells compared with NTC control after oleic acid challenge. Assessment of cell viability after the Nile Red binding assays with Resazurin showed no significant difference between the cells treated with FABP5 siRNA/oleic acid and NTC siRNA-treated cells ($n=3$ independent experiments; data not shown), suggesting that the increase in Nile Red binding in FABP5 siRNA/oleic acid-treated cells was not associated with cell death, and thus reflected a true increase in lipid deposits.[4]

Decreased Secretion of apoB100 by FABP5 Knockdown

The availability of cellular cholesterol can influence apoB lipoprotein particle production and secretion.[5–7] We first used western blot analysis to determine whether apoB protein is secreted into the culture medium, and whether FABP5 inhibition influences this secretion. We found a 56% reduction of ApoB in the culture medium after FABP5 siRNA treatment when compared with NTC controls. In contrast, the amount of total secreted protein in the cultured medium from FABP5 siRNA- and NTC-treated cells is comparable under our experimental conditions. To determine whether the apoB protein identified by western blot analysis is synthesized and secreted and not a contaminant of the culture medium, we next evaluated apoB100 secretion using ^{35}S-labeled apoB100 immunoprecipitation. We isolated ^{35}S-labeled apoB100 at 520 kDA and its degraded products with apparent molecular weights of 250 and 75 kDa from the cultured medium. We found that the level of newly synthesized apoB in the culture medium from FABP5 siRNA-treated cells was decreased by 76% ($P<0.05$) than that from NTC siRNA-treated cells (Figure 5). This result correlates nicely with the western blot results, and suggests that apoB100 is not a contaminant of the medium.

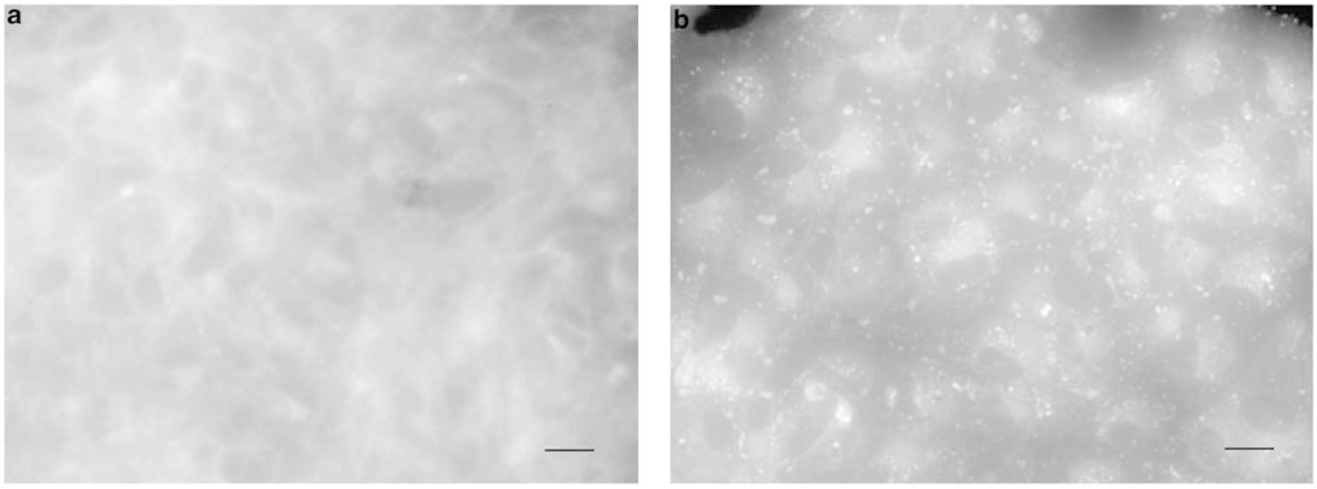

Figure 4 Nile Red fluorescence microscopy. ARPE-19 cells were treated with 10 nM siRNA for 6 h, then in medium containing 1% BSA for 24 h. Nile Red assay was performed after cells incubated in medium supplemented with 0.4 mM oleic acid for an additional 24 h. (**a**) NTC-treated cells show few cellular lipid droplets compared with treatment with an siRNA to FABP5 (**b**). Nuclei appear dark in the image. Bar = 10 μm.

DISCUSSION

The following paragraphs are amended as follows:

Paragraph 2

This study has shown that knockdown of the *FABP5* gene results in several significant changes in ARPE-19 cells: (1) altered cellular lipid composition, (2) an increase in cellular lipid droplets, and (3) decreased secretion of apoB100. As a principal function of *FABP5* is to transport FAs in the cytoplasm to organelles, such as the endoplasmic reticulum, the lack of FABP5 protein may disrupt the intracellular transport of FAs, resulting in an 18 % increase in cellular levels of FFA in FABP5 siRNA-treated cells compared with that in NTC siRNA-treated cells (Table 1). Such a cellular accumulation of FAs can potentially alter lipolysis and lipogenesis in FABP5 siRNA-treated cells. *FABP5* appears to facilitate FFA efflux from cells or some type of transfer of lipid from storage droplets to secretory droplets. As most triglycerides are found in lipid droplets within the cytosol, and are not associated with the endoplasmic reticulum, triglycerides must be mobilized by lipolysis, followed by reesterification, for triglycerides to be reassembled in the endoplasmic reticulum before being incorporated into lipoprotein particles for secretion.[8,9] Importantly, these processes may have been interrupted by the lack of *FABP5*, causing a decrease in the efficacy of FA release or blocking the reesterification of liberated FAs in the endoplasmic reticulum. In contrast, overexpression of *FABP5* has been reported to increase lipolysis in adipose cells.[10]

Paragraph 5

The decreased cholesterol and cholesterol ester composition that we observed as a result of *FABP5* gene knockdown led us to examine the secretion of apoB100 in FABP5 siRNA-treated cells. The apoB-containing lipoprotein assembly/secretion pathway is dependent on (1) the production of apoB, which is necessary for the assembly of lipoprotein particles containing a neutral lipid core; (2) the availability of phospholipids (mainly PC), free cholesterol to form the monolayer surface and triglycerides, and cholesterol ester to form the core of the particles; and (3) the availability of microsomal triglyceride transfer protein (MTP).[11] In the absence of any one or more of these factors, apoB is diverted from the lipoprotein particle assemble/secretion pathway and is degraded. In HepG2 liver cells, cholesterol ester reduction has been shown to reduce the secretion of apoB-containing lipoproteins significantly.[12] Consistent with these findings, our study data suggest that the decreased secretion of apoB100 (Figure 6) could have been caused by a disruption in the formation of apoB100-containing lipoprotein-like particles, as a result of a substantial decrease in cholesterol and cholesterol esters after *FABP5* knockdown. The lower levels of some species of phospholipids might also contribute to the decreased secretion of apoB in siRNA-treated cells (Table 2).

Paragraph 6

Our experiments suggest that knockdown of the *FABP5* gene decreased cellular cholesterol, cholesterol ester, and some species of phospholipids, the main constituents of lipoprotein-like particles. As a consequence, it may also reduce the production of apoB-containing lipoproteins and lead to decreased secretion of apoB as well as the accumulation of cellular FFAs and triglycerides. Using a knockdown approach, we were able to observe dramatic and unexpected phenotypic changes in FABP5 siRNA-treated ARPE-19 cells. These observations suggest that FABP5 may be a key FA transporter and is critical in lipid metabolism in human ARPE-19 retinal pigment epithelial cells. Given the decrease in FABP5 expression by the RPE/choroid complex in a murine model

of early AMD,[1] the findings from this study in turn, imply a possible scenario in which the downregulation of FABP5 in the RPE/choroid complex *in vivo* might contribute to age-related changes and early AMD.

All the authors now agree that the data and the interpretations are accurate.

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