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Adipose tissue depot-specific differences in adipocyte apolipoprotein E expression

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ABSTRACT

Important differences in gene expression have been documented in adipocytes derived from specific adipose tissue depots. We have previously documented an important role for adipocyte apolipoprotein E (apoE) in modulating adipocyte and adipose tissue triglyceride and lipoprotein metabolism. We now evaluate the endogenous expression of apoE in adipocytes isolated from unique adipose tissue depots in 4 different species. Adipocyte apoE expression is higher in subcutaneous fat compared with visceral fat in humans, mice, rats, and baboons. In baboons, evaluation of apoE expression in 5 adipose tissue depots (subcutaneous abdominal, subcutaneous gluteal, visceral, pericardial, epicardial) showed that, compared with subcutaneous abdominal adipocytes, the level of apoE expression is similar in subcutaneous gluteal, lower in visceral and pericardial, and higher in epicardial adipocytes. Consistent with previously demonstrated suppression of adipocyte apoE by adipose tissue inflammation, adipose tissue depots with lower apoE expression demonstrated greater infiltration of macrophages and an increased expression of tumor necrosis factor–α messenger RNA. Depot-specific differences in apoE expression were maintained after in vitro differentiation. Adipocytes isolated from depots with lower apoE expression manifested lower rates of triglyceride synthesis in the absence and presence of triglyceride-rich lipoproteins. Adenoviral-mediated increase of apoE expression in omental adipocytes increased triglyceride synthesis in these cells. Our results demonstrate significant heterogeneity in adipocyte apoE expression across adipose tissue depots in several species. Because of its role in modulating adipocyte triglyceride and lipoprotein metabolism, depot-specific differences in endogenous adipocyte apoE could have important implications for modulating the accumulation of lipid in these depots.

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1. Introduction

The increasing prevalence of obesity produces an increasing population risk for significant metabolic and cardiovascular complications [1-4]. Significant evidence has accrued indicating heterogeneity of adipose depots with respect to producing risk for these complications [2-6]. For example, visceral adipose tissue (as opposed to subcutaneous adipose tissue) has been more closely related to systemic inflammation, glucose dysregulation, and atherosclerotic vascular disease risk [2-6]. Results from a number of studies have also suggested that subcutaneous adipose tissue, particularly that in the lower body, can act as a sink to store and sequester excess calories, prevent its ectopic deposition, and thereby minimize cardiovascular and metabolic risk [7-9]. Altered expression of genes that could influence metabolic or cardiovascular complications has also been demonstrated in other fat depots, for example, epicardial fat [10-12].

We have recently demonstrated an important role for endogenous adipocyte apolipoprotein E (apoE) expression in modulating adipose tissue and adipocyte substrate metabolism and storage [13-15]. Apolipoprotein E, which has long been established as a surface constituent of circulating very low-density lipoprotein (VLDL) and high-density lipoprotein particles in humans, was first described as an important endogenous product of adipocytes more than 2 decades ago [16]. More recently, we have described several physiologically relevant pathways for regulating adipocyte apoE expression using in vitro and in vivo models. Physiologic regulators of adipocyte apoE expression include thiazolidinedione drugs and other peroxisome proliferator–activated receptor (PPARγ) agonists, inflammatory cytokines, and reactive oxidant species [17-21]. The onset of obesity produces substantial down-regulation of adipocyte apoE expression, and this down-regulation is mediated by inflammatory cytokines and reactive oxygen species produced by adipose tissue stromovascular macrophages [22].

We have also investigated the importance of endogenous adipocyte apoE expression for the differentiated function of these cells. Using both in vitro and in vivo models, we have produced substantial evidence that endogenous adipocyte apoE expression importantly influences adipocyte substrate metabolism and gene expression [13-15]. Moreover, these functions of endogenous adipocyte apoE cannot be substituted by the provision of exogenous apoE. This latter point is made most dramatically in studies using adipose tissue transplantation from apoE knockout (EKO) to wild-type (WT) mice. Fourteen weeks after transplantation of EKO or WT adipose tissue into WT mice, EKO adipocytes in transplanted adipose tissue are smaller and triglyceride (TG) poor, and display a defect in TG synthesis compared with WT adipocytes harvested from transplanted WT adipose tissue [14]. This result demonstrates that, despite being exposed to WT levels of circulating apoE in TG-rich lipoproteins in vivo for 14 weeks, the lack of endogenous adipocyte apoE expression produces a defect in adipocyte substrate acquisition. With respect to a mechanism for this defect, we have also shown that absence of endogenous apoE expression in adipocytes markedly impairs the acquisition of fatty acid substrate from extracellular TG-rich lipoprotein particles and thereby impairs accumulation of TG in EKO adipocytes [15].

In view of the role we have established for endogenous apoE expression in adipose tissue and adipocyte TG metabolism, and the emerging role of specific adipose tissue depots for modulating systemic metabolism and cardiometabolic risk, we undertook a series of studies to evaluate the level of apoE expression in distinct adipose tissue depots. For these studies, we evaluated multiple species and investigated obese and nonobese experimental models. We further investigated the relationship between apoE expression and TG metabolism in adipocytes isolated from specific depots.

2. Methods

2.1. Materials

All chemicals were from Sigma (St Louis, MO). Cell culture medium and fetal bovine serum were purchased from Invitrogen (Carlsbad, CA). Organic solvents were from Fisher (Pittsburgh, PA). [^{14}C]glucose was obtained from PerkinElmer Life Sciences (Waltham, MA). Very low-density lipoprotein was isolated by sequential density gradient ultracentrifugation of human plasma as previously described in detail [13]. A goat anti-apoE antibody was purchased from International Immunology (Marietta, GA).

2.2. Human study subjects

Matched abdominal subcutaneous and visceral adipose tissue samples were obtained from 17 female adult and adolescent patients undergoing bariatric surgery or hernia repair surgery at the University of Illinois at Chicago (UIC) Medical Center. All subjects gave their informed consent to the study, which was approved by the Institutional Review Board at UIC.

2.3. Baboon study subjects

Baboons (*Papio anubis*) (n = 6, female) used in this study were from the UIC primate colony containing control animals for other studies approved by the UIC Institutional Animal Care and Use Committee. Adipose tissue was harvested from euthanized animals and immediately preserved for isolation of mature adipocytes, isolation of preadipocytes, or isolation of RNA [13].

2.4. Mouse and rat adipose tissue

C57BL/6 mice (12 weeks old, male) were purchased from Charles River (Wilmington, MA) and maintained on either chow diet (13.4% kcal from fat; Harlan Teklad, Madison, WI) or high-fat diet (HFD) (60% kcal from fat; Harlan Teklad) for 12 weeks. Male 9- to 10-week-old Zucker fatty rats (*fa/fa*) or lean littermates (*fa/+*) were purchased from Charles River. All animal protocols were approved by the Institutional Animal Care and Use Committees of UIC.

2.5. Adipose tissue handling and adipocyte isolation and differentiation

Adipose tissue for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis was flash frozen in liquid nitrogen. Mature floating adipocytes were isolated from freshly harvested adipose tissues for immediate use as previously described [13]. Preadipocytes from the adipose tissue stromovascular fraction were isolated and differentiated into adipocytes using a 3-day incubation in insulin, dexamethasone, isobutyl methylxanthine, and rosiglitazone as previously described in detail [13-15]. Cells differentiated in culture were used for experiments 10 to 14 days later. Adenoviral transduction was used to increase endogenous cellular apoE expression as previously described [13]. Briefly, rat or human adipocytes harvested from the omental adipose tissue depot were incubated with adenovirus directing the expression of LacZ (as a control) or apoE at a multiplicity of 25 for 6 hours. After 4 additional days in complete growth medium, cells were used for experiments.

2.6. qRT-PCR analysis for messenger RNA levels

Quantitative RT-PCR was performed as previously described [13-15]. For normalization, human, baboon, and mouse RNA samples were standardized to 3 housekeeping genes using the geNorm program [23]. β-Actin was used for normalization of rat RNA samples. The primer sets used for this study are listed in Table 1. Data were analyzed using the comparative critical threshold method. Fold change was calculated by $2^{-\Delta\Delta Ct}$.

2.7. TG synthesis and mass

Cultured adipocytes were incubated with 0.5 μCi/mL [^{14}C] glucose with or without 100 μg/mL VLDL in Dulbecco modified Eagle medium and 0.1% bovine serum albumin for 6 hours at 37°C. After washing, cellular lipids were extracted; and TGs were separated by thin layer chromatography in a solvent system of hexane:ethyl ether:acetic acid (90:30:1). The TG spots were harvested, and radioactivity in spots was measured in a scintillation counter. Cell protein was estimated using the Bio-Rad DC protein kit (Hercules, CA). Cellular TG mass was measured using a kit obtained from Wako (Richmond, VA) as previously described [13].

2.8. Western blot

Adipocytes were lysed, and protein extracts were prepared for apoE Western blot analysis as previously described in detail [13-15]. Western blot images were quantitated using ImageQuant TL software (GE Healthcare, Piscataway, NJ) and corrected for actin as an internal loading control.

2.9. Statistics

Results are shown as mean ± SD as described in figure legends. Statistical differences were analyzed by Wilcoxon signed rank test for human studies and by *t* test or analysis of variance for baboon, rodent, and in vitro studies using SPSS 18.0 (Chicago, IL). $P < .05$ was considered significant.

3. Results

3.1. ApoE expression in visceral compared with subcutaneous adipocytes in obese humans

Apolipoprotein E messenger RNA (mRNA) level was measured in adipocytes isolated from freshly harvested adipose tissue from 17 adolescent and adult female subjects undergoing bariatric surgery or hernia repair surgery at UIC. The characteristics of the subjects are shown in Table 2. Subjects were female with a median age of 30 years (range, 14-55 years). Median body weight was 130.9 kg, and median body mass index was 48.0. Comorbidities included diabetes and hyperlipidemia. One subject was a current smoker. Fig. 1 shows

Table 1 – PCR primer sequences

Species	Gene	Forward	Reverse
Homo sapiens	ApoE	CACAGGCAGGAAGATGAAGGT	AGCGCAGGTAATCCCAAAAG
	LRP10	AGTGGGACTGCTCCTATGTTCT	CACAATCTCAGCCTCCATCC
	β-Actin	ACTCTTCCAGCCTTCCTTCCT	CAGTGATCTCCTTCTGCATCCT
	HPRTI	CTGAGGATTTGGAAAGGGTGT	AATCCAGCAGGTCAGCAAAG
P anubis	ApoE	CTCTTGGTTCACTGCCCTTCT	TTCAACTCCTTCATGGTCTCGT
	CD68	CGAGCATCATTCTTTCACCA	AGGAGGCCAAGAAGAACCA
	TNF	TCTTCACTGGAAAGGACACCA	GAAGGAGAAGAGGCTGAGGAA
	β-Actin	ATCGTGCGTGACATTAAGGAG	CCAGGAAGGAAGGTTGGAAG
	Cyclophilin A	CAAGACGGAGTGGTTGGATG	TGGTGGTCTTCTTGCTGGTC
	GAPDH	AGCGAGATCCCTCCAAAATC	GTTCACACCCATGACGAACA
Mus musculus	ApoE	AGTGGCAAAGCAACCAACC	CTTCCGTCATAGTGTCCTCCA
	β-Actin	CTGGGACGACATGGAGAAGA	AGAGGCATACAGGGACAGCA
	36B4	AGCGCGTCCTGGCATTGTGTGG	GGGCAGCAGTGGTGGCAGCAGC
	Cyclophilin A	TGGTCTTTGGGAAGGTGAAAG	TGTCCACAGTCGGAAATGGT
Rattus norvegicus	ApoE	ATCAGCTCCCAGGGCAAA	TTCCGTCATAGTGTCCTCCATC
	β-Actin	CTGGGACGACATGGAGAAGA	AGAGGCATACAGGGACAGCA

LRP10 indicates low-density lipoprotein receptor–related protein 10; HPRT1, hypoxanthine phosphoribosyl-transferase 1; 36B4 or RPLP0, acidic ribosomal phosphoprotein P0 cyclophilin A.

Table 2 – Characteristics of human subjects

	Median (range)
N	17
Age (y)	30.0 (14.0-55.0)
Sex	Female
Ethnicity	White (9/17) Hispanic (3/17) African American (5/17)
Body weight (kg)	130.9 (102.3-204.5)
BMI	48.0 (34.4-62.8)
Diabetes	4
Hyperlipidemia	6
Smoking	1

BMI indicates body mass index.

apoE mRNA level in adipocytes harvested from subcutaneous or visceral adipose tissue presented as a box plot of the cycle threshold number for apoE compared with the cycle threshold number for the housekeeping genes (figure legend and "Methods"). Based on the difference in cycle number, adipocytes from visceral adipose tissue expressed 0.71 ± 0.26 of the apoE mRNA level found in adipocytes isolated from subcutaneous adipose tissue ($P < .05$).

3.2. *ApoE expression in subcutaneous and intraabdominal fat harvested from lean and obese rodents*

Adipocytes were harvested from adipose tissue obtained from the subcutaneous or omental depots of Zucker fatty rats or lean littermates (Fig. 2). Lean and obese rats weighed 225 to 250 g and 350 to 375 g, respectively. Apolipoprotein E mRNA levels were approximately 6-fold higher in subcutaneous compared with visceral adipocytes harvested from lean rats. With obesity, the difference between visceral and subcutaneous fat was reduced. However, subcutaneous adipocytes demonstrated 2- to 3-fold higher levels of apoE mRNA compared with omental adipocytes.

Fig. 3 presents results for apoE mRNA and protein levels in subcutaneous compared with intraabdominal (epididymal fat pad) adipocytes isolated from chow-fed mice or mice maintained on an HFD. At time of harvest, body weight for chow-fed mice was 33 ± 4 g; and for those maintained on HFD, body weight was 50 ± 2 g. For both chow-fed and high-fat–fed mice, apoE mRNA (Fig. 3A) and protein (Fig. 3B) were significantly higher in adipocytes isolated from the subcutaneous fat depot. Fig. 3C demonstrates suppression of apoE gene expression in each depot in response to the HFD. Interestingly, suppression in visceral adipocytes tended to be greater.

3.3. *ApoE expression in adipose tissue and adipocytes harvested from nonhuman primates*

We next evaluated apoE expression in adipose tissue and adipocytes isolated from a nonhuman primate model. This model afforded the opportunity to evaluate apoE expression in multiple adipose tissue depots and provided sufficient adipose tissue to allow comparison of apoE expression between freshly isolated adipocytes and cultured adipocytes. The characteristics of the baboons used in this study are shown in Table 3. Fig. 4 shows levels of apoE, tumor necrosis factor (TNF) α, and CD68 mRNA in adipose tissue. The level of expression in omental, subcutaneous gluteal, pericardial, and epicardial adipose tissue is presented relative to that found in subcutaneous abdominal adipose tissue. For each panel, therefore, the level of expression of apoE, TNFα, and CD68 in subcutaneous abdominal tissue is assigned a value of 1 (represented by the hatched line). In adipose tissue isolated from the omental depot, apoE expression is reduced by approximately 50%, whereas the expression of both CD68 (a marker of macrophage infiltration) and TNFα (an inflammatory cytokine we have previously shown to downregulate adipocyte apoE expression) is elevated by about 2-fold. There are no substantial differences in apoE, TNFα, and CD68 expression between subcutaneous gluteal and subcutaneous abdominal tissue. The pattern of expression for pericardial tissue is similar to that observed in omental adipose tissue with reduced apoE expression, increased TNFα, and increased CD68 expression compared with subcutaneous abdominal tissue. On the other hand, epicardial adipose tissue has increased levels of apoE expression compared with subcutaneous abdominal tissue with reduced expression of TNFα and a trend toward reduction in CD68 expression.

Fig. 5 shows level of apoE expression in mature adipocytes studied immediately after isolation from freshly harvested adipose tissue and that in adipocytes differentiated from preadipocytes in vitro and maintained in culture for 10 to 14 days. The level of apoE expression in freshly isolated mature omental, subcutaneous gluteal, pericardial, and epicardial adipocytes is compared with that in freshly isolated mature subcutaneous abdominal adipocytes. The level of apoE

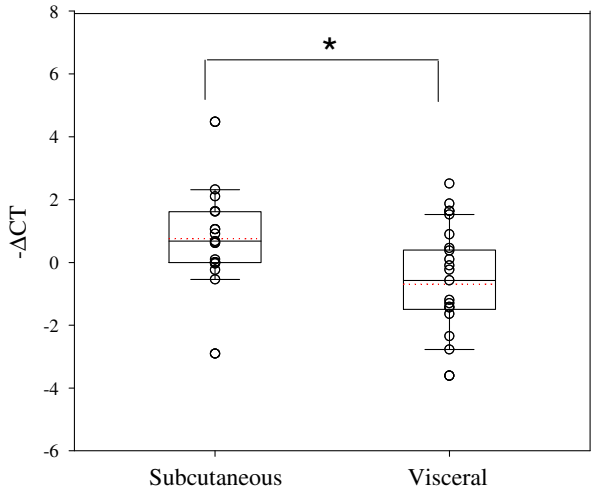

Fig. 1 – Apolipoprotein E mRNA levels in human subcutaneous and visceral mature adipocytes. Mature adipocytes were isolated from matched sets of freshly harvested subcutaneous and visceral adipose tissue depots (n = 17) as described in "Methods." Apolipoprotein E mRNA levels were measured by qRT-PCR. Results presented in the box plot graph are ΔCT number of subcutaneous and visceral samples as described in "Methods." Each sample was run in duplicate. *P < .05 for the difference in apoE mRNA level between subcutaneous and visceral adipocytes.

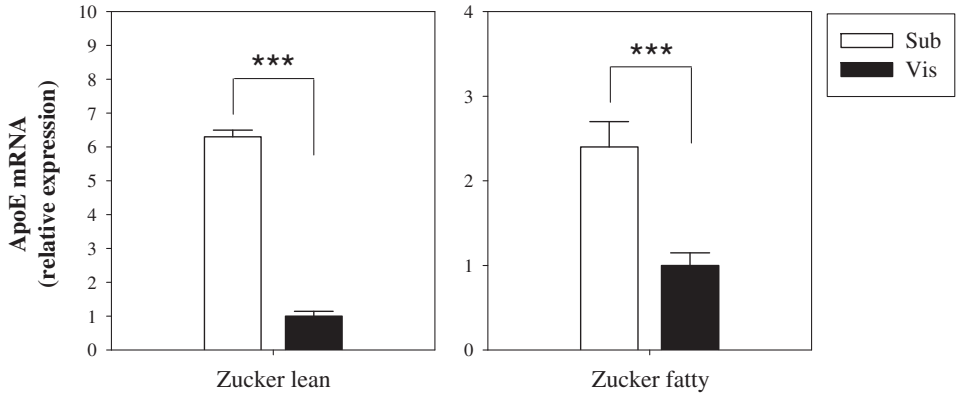

Fig. 2 – Apolipoprotein E mRNA levels in rat subcutaneous and omental mature adipocytes. Mature adipocytes were isolated from freshly harvested subcutaneous or omental fat depots from Zucker fatty rats or lean littermates. Apolipoprotein E mRNA levels were measured by qRT-PCR. Results shown are mean ± SD of 3 rats per group. ***P < .001 for the difference in apoE mRNA level between subcutaneous and omental adipocytes.

expression in cultured omental, subcutaneous gluteal, pericardial, and epicardial adipocytes is compared with that in cultured subcutaneous abdominal adipocytes. Consistent with our observations in whole adipose tissue, apoE expression is substantially decreased in isolated mature adipocytes freshly harvested from omental compared with the subcutaneous adipose tissue depot. This reduction in apoE expression in omental adipocytes is maintained even when cells have been differentiated from preadipocytes in culture and maintained in culture for 10 to 14 days. Likewise, the relationship of apoE expression in subcutaneous gluteal, pericardial, and epicardial mature adipocytes compared with that in subcutaneous mature adipocytes is maintained in the cultured adipocytes.

We have previously reported changes in gene expression in cultured EKO compared with WT adipocytes. The expression of acetyl-CoA dehydrogenase, medium chain; PPARγ coactivator; carnitine palmitoyltransferase–1; and acyl-CoA oxidase was higher and expression of adiponectin, CCAAT/enhancer binding protein, PPARγ, and caveolin was lower when comparing EKO to WT adipocytes. Similar, but smaller, changes were observed in gene expression comparing visceral (with lower apoE gene expression) to subcutaneous (with higher apoE gene expression) cultured adipocytes. The level of expression of acetyl-CoA dehydrogenase, medium chain (1.5 ± 0.4); PPARγ coactivator (2.0 ± 0.3); and carnitine palmitoyltransferase–1 (1.9 ± 0.7) was significantly higher and the expression of CCAAT/enhancer binding protein (0.4 ± 0.3), PPARγ (0.3 ± 0.2), and caveolin (0.4 ± 0.2) was significantly lower when comparing visceral to subcutaneous cultured adipocytes.

3.4. *Implications of depot-specific differences in apoE expression for adipocyte TG homeostasis*

The above information clearly establishes significant differences in apoE expression across adipose tissue depots in 4 different species. Results previously published from our laboratory suggest that these differences should impact adipocyte TG metabolism [13-15]. We addressed this question using adipocytes isolated from the baboon model. Preadipocytes were isolated from the subcutaneous abdominal or omental depots of 2 different baboons, and the rate of TG synthesis was measured (as described in "Methods") in the absence or presence of VLDL. The latter serves as a source of substrate driving adipocyte TG synthesis, and we have previously shown that adipocyte TG synthesis measured in both the presence and absence of VLDL is lower in adipocytes with reduced apoE expression [13-15]. Consistent with the reduced apoE expression in omental adipocytes, TG synthesis is significantly lower in omental compared with subcutaneous adipocytes in both the presence and absence of VLDL (Fig. 6A). To confirm that decreased TG synthesis in omental adipose tissue was related to decreased endogenous adipocyte apoE expression level, we used an apoE-expressing adenovirus to increase apoE expression in adipocytes cultured from omental adipose tissue in humans and rats (Fig. 6B). A LacZ adenovirus was used as control. In multiple experiments, transduction with the apoE adenovirus led to a 4- to 10-fold increase in apoE expression compared with the LacZ adenovirus. Increased expression of apoE in omental adipocytes from both species was associated with a significant increase in adipocyte TG content after incubation in lipoprotein-containing growth medium.

4. Discussion

The results in this report establish that adipocytes from the visceral fat depot express lower levels of apoE compared with those from the subcutaneous abdominal fat depot in humans, mice, rats, and baboons. Depot differences are significant in obese humans but are somewhat smaller than in the animal experimental models, perhaps related to less marked genetic heterogeneity present in the animal models. In rats and mice, we demonstrate that depot-specific differences are maintained in obesity. In baboons, we demonstrate that, compared with subcutaneous abdominal fat, there is no difference in apoE expression in subcutaneous gluteal fat, but that apoE expression in pericardial fat is lower (similar to visceral fat) but higher in epicardial fat. Provocatively, results in freshly

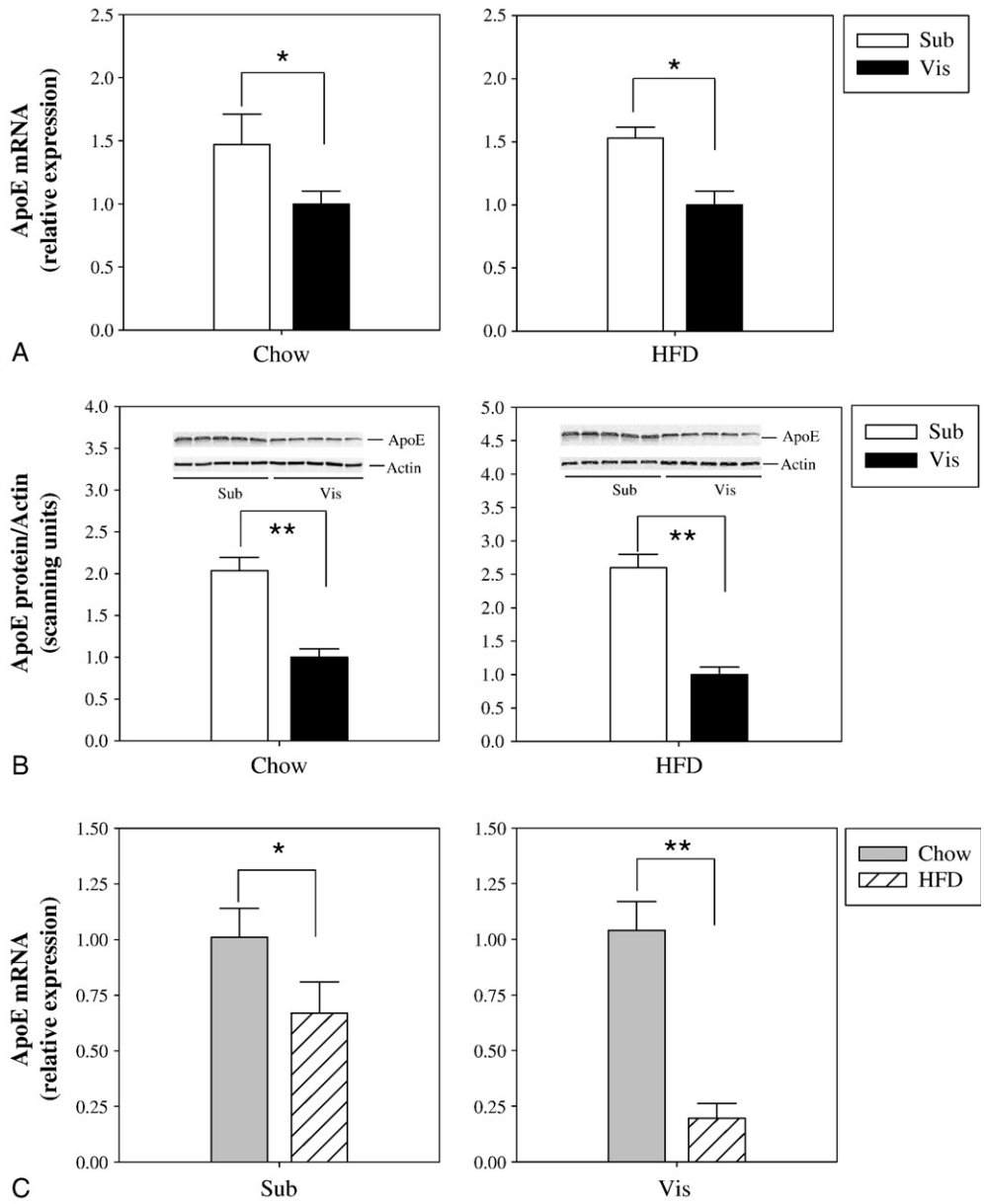

Fig. 3 – Apolipoprotein E mRNA and protein expression in mouse subcutaneous and intraabdominal mature adipocytes. Mature adipocytes were isolated from freshly harvested subcutaneous and visceral fat depots from chow-fed or high-fat–fed (12 weeks) mice (n = 6-8 mice per group). A, Apolipoprotein E mRNA levels were measured by qRT-PCR, and (B) ApoE protein expression was estimated by Western blot. Apolipoprotein E and ß-actin signals are shown from the Western blot of samples from 5 different mice. C, Changes in apoE expression in visceral and subcutaneous adipocytes with HFD-induced obesity. *P < .05, **P < .01 for the indicated comparison.

Table 3 – Characteristics of study baboons *(Papio Anubis)*

	Median (range)
N	6
Sex	Female
Body weight (kg)	20.2 (17.5-32.1)
Diabetes	No
Medications	No
Hyperlipidemia	No
Smoking	No

isolated mature adipocytes compared with those in adipocytes differentiated from preadipocytes in vitro indicate that precursor cells are already committed to depot-specific differences in apoE expression.

We have previously shown that reactive oxygen species or TNFα from macrophages in the adipose tissue stromovascular fraction can significantly reduce adipocyte apoE expression [22]. It seems reasonable, therefore, to assume that the lower levels of adipocyte apoE measured in visceral and pericardial adipose tissue depots could result from the increased macrophage infiltration (as marked by increased CD68 expression)

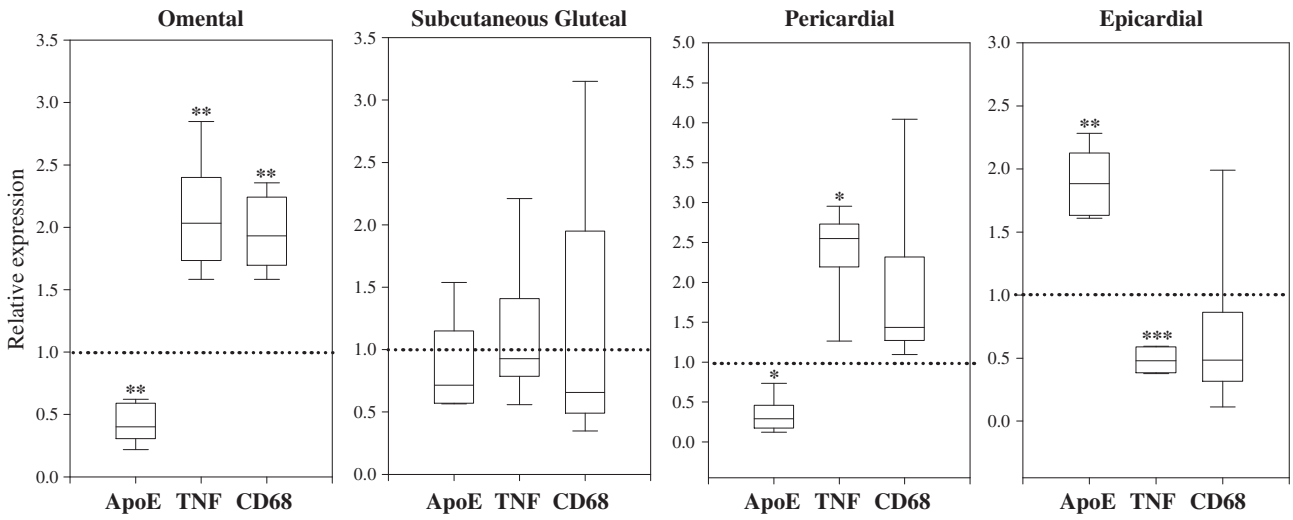

Fig. 4 – Evaluation of apoE, CD68, and TNFα levels in 5 adipose tissue depots from the baboon. Subcutaneous abdominal, omental, subcutaneous gluteal, pericardial, and epicardial adipose tissue was harvested from 6 baboons. Apolipoprotein E, CD68, and TNF mRNA levels were measured by qRT-PCR. Expression levels in omental, subcutaneous gluteal, pericardial, and epicardial adipose tissue depots are expressed in box plot format relative to expression in subcutaneous abdominal, which is represented by the dashed line at "1" in each panel. Results are presented as fold change. *P < .05, **P < .01, ***P < .001 for the difference compared with the subcutaneous abdominal fat depot.

and an increased inflammatory milieu (as marked by increased TNFα expression) we detect in these depots. However, our results also demonstrate that depot-specific differences in adipocyte apoE expression are maintained in precursor cells that have divided, undergone differentiation, and been maintained in culture for 14 days. This observation suggests that adipocyte precursor cells in unique adipose tissue depots are already committed to a specific level of apoE expression. This observation is consistent with those of others showing that depot-specific differences in adipocyte gene

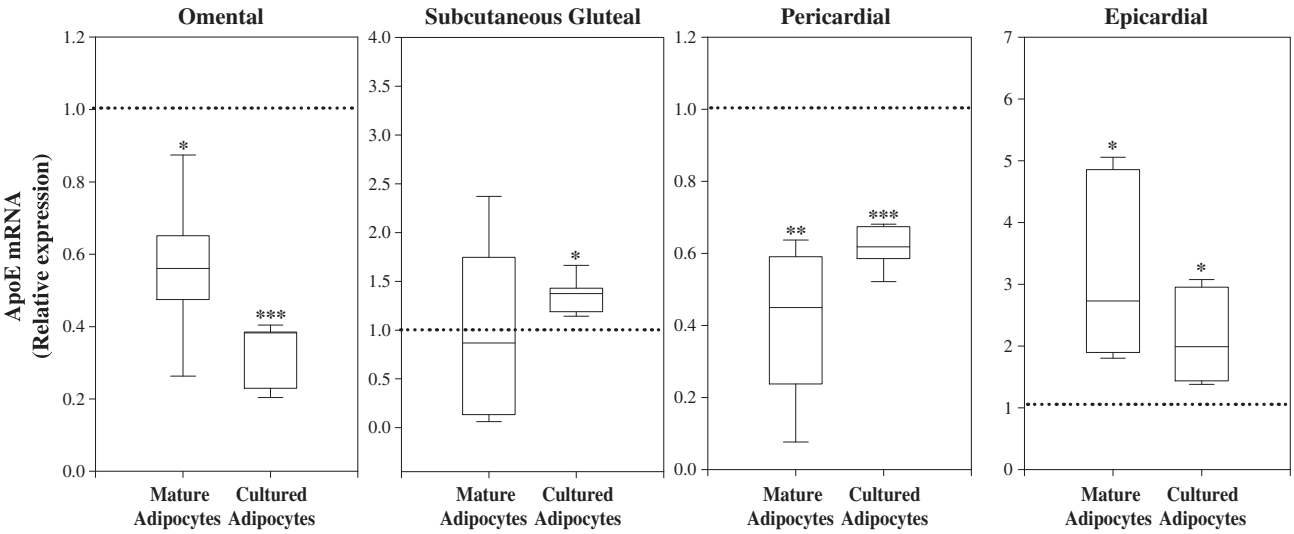

Fig. 5 – Apolipoprotein E expression in freshly isolated mature adipocytes compared with cultured adipocytes. RNA was isolated from mature adipocytes isolated from the 5 adipose tissue depots noted in Fig. 4. RNA was also isolated from adipocytes differentiated from preadipocytes isolated from the same 5 depots, and maintained in culture for 10 to 14 days. Apolipoprotein E mRNA level was measured by qRT-PCR, and its level of expression in freshly isolated mature adipocytes in each depot was compared with that measured in freshly isolated mature adipocytes from the subcutaneous abdominal fat depot. The level of expression in cultured adipocytes from each depot was compared with that in cultured adipocytes from the subcutaneous abdominal fat depot. Results are presented in box plot format as fold changes, with the dashed line at "1" representing level of expression in the mature adipocytes or cultured adipocytes from the subcutaneous abdominal depot. *P < .05, **P < .01, ***P < .001 for the difference in expression level compared with mature adipocytes or cultured adipocytes isolated from the subcutaneous abdominal fat depot.

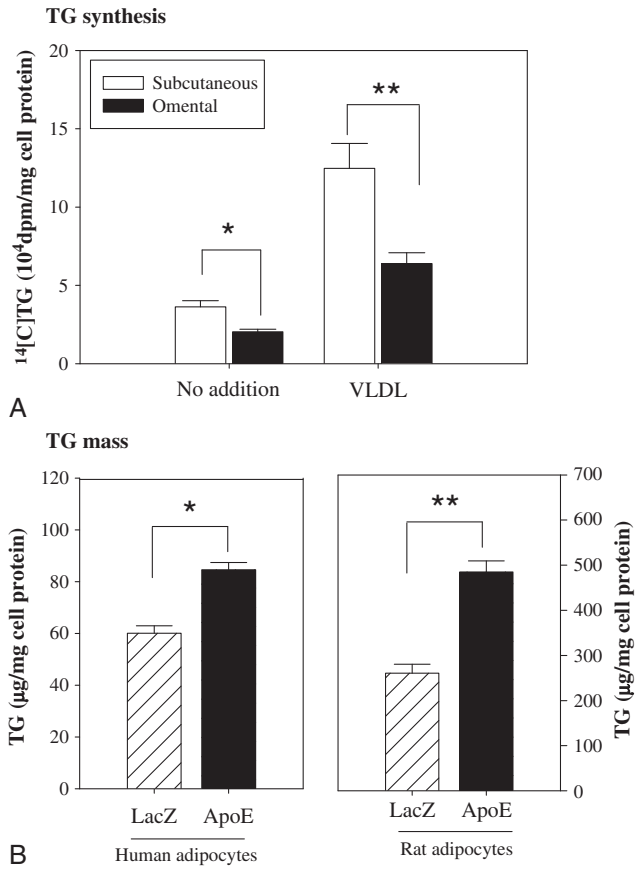

Fig. 6 – Apolipoprotein E expression and TG synthesis/mass. A, Baboon adipocytes were prepared from preadipocytes isolated from omental and subcutaneous adipose tissues and maintained in culture for 14 days as described in "Methods." To measure TG synthesis, adipocytes were incubated with no addition or with 100 μg/mL VLDL in Dulbecco modified Eagle medium containing [^{14}C]glucose and 0.1% bovine serum albumin for 6 hours. Triglyceride synthesis over 6 hours was measured as described in "Methods." Results presented are mean of experiments from 2 separate baboons, each run in triplicate. *P < .05, **P < .01 for the difference between subcutaneous and omental adipocytes. B, Rat or human cultured adipocytes from the omental adipose tissue depot were incubated with an apoE or LacZ adenovirus as described in "Methods." After an additional 4 days of incubation in lipoprotein-containing growth medium, cells were harvested for measurement of TG mass. Results shown are mean of 2 separate experiments, each ran in triplicate. *P < .05, **P < .01 for the indicated comparison.

expression are also maintained in long-term culture for other genes including adiponectin and genes involved in insulin signaling [24]. In fact, preadipocyte strains derived from single preadipocytes have been shown to maintain depot-specific cell characteristics after 40 divisions in culture [25,26]. In the case of apoE expression, the long-term exposure to differing adipose tissue inflammatory milieu (Fig. 4) could play a role in imprinting preadipocyte precursors.

Our previous work has established that endogenous adipocyte apoE expression has important implications for the acquisition of substrate from TG-rich lipoprotein particles like VLDL [14]. Apolipoprotein E knockout adipocytes are lipid poor, and TG synthesis is lower compared with WT adipocytes in the absence and presence of extracellular VLDL. We have also previously shown that the difference in TG synthesis between EKO and WT adipocytes can be corrected by adenoviral expression of apoE in EKO adipocytes [13]. Based on the results of these prior studies, adipocytes derived from depots with lower expression of apoE would be predicted to manifest lower levels of TG synthesis in the absence and presence of VLDL (Fig. 6A); and increasing the expression of apoE in adipocytes harvested from depots with low apoE expression should increase acquisition of substrate and TG mass (Fig. 6B). Although our study does not address depot-specific differences in apoE expression as they relate to sex, it is interesting to speculate that a sex effect could contribute to the well-established differences in adipose tissue distribution between human males and females.

Many examples of differential gene expression in cells derived from subcutaneous vs visceral fat have been documented, along with important systemic implications deriving from this differential expression. Depot-specific effects for gene expression in pericardial and epicardial depots have more recently come to attention [10-12]. Cross-sectional associations between altered gene expression in pericardial and epicardial fat and the presence of human disease (ie, coronary atherosclerosis or obesity) have also recently been reported [10-12]. Our results establish a depot-specific influence on apoE

expression level in pericardial and epicardial adipose tissue depots. Apolipoprotein E expression in the pericardial depot is lower than that in subcutaneous fat, similar to that observed in visceral fat. However, apoE expression in epicardial adipose tissue is higher than that observed in any depot examined. An interesting potential implication of apoE gene expression in epicardial fat relates to the absence of a fascia between the epicardial fat pad and the epicardial coronary arteries, the site of atherothrombotic disease that gives rise to clinically important myocardial ischemia. There are several well-established mechanisms by which apoE secreted from the epicardial fat could favorably impact the evolution of atherothrombotic disease in the coronary arteries [27-29].

In summary, our results demonstrate significant heterogeneity in the expression of adipocyte apoE across adipose tissue depots in several species. These differences in adipocyte apoE expression have implications for adipocyte TG and VLDL metabolism, as predicted by the results of prior in vitro and in vivo mechanistic studies. Some differences in adipocyte apoE expression across adipose tissue depots could be related to depot-specific differences in macrophage infiltration and inflammatory microenvironment, as we have already demonstrated that TNFα and reactive oxygen species produced by adipose tissue macrophages can significantly suppress adipocyte apoE expression. Because of the important role that has been established for endogenous adipocyte apoE expression in overall adipose tissue substrate flux, the depot-specific changes we currently demonstrate in adipocyte apoE expression could have important implications for modulating the accumulation of TG in these depots.

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Conflict of Interest

The authors have nothing to disclose.

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