RESEARCH ARTICLE

CD36 is involved in lycopene and lutein uptake by adipocytes and adipose tissue cultures

Myriam Moussa[1,2], Erwan Gouranton[1,2], Béatrice Gleize[1,2,3], Claire El Yazidi[1,2], Isabelle Niot[4], Philippe Besnard[4], Patrick Borel[1,2,3] and Jean-François Landrier[1,2]

[1] INRA, UMR1260 ''Nutriments Lipidiques et Prévention des Maladies Métaboliques'', Marseille, France
[2] University Aix-Marseille 1, University Aix-Marseille 2, Faculté de Médecine, Marseille, France
[3] INSERM, ERL U1025''Bioavailability of micronutrients'', Marseille, France
[4] Physiologie de la Nutrition, UMR INSERM U 866, AgroSup Dijon, Université de Bourgogne, Dijon, France

Received: August 24, 2010
Revised: October 11, 2010
Accepted: October 22, 2010

Scope: Carotenoids are mainly stored in adipose tissue. However, nothing is known regarding the uptake of carotenoids by adipocytes. Thus, our study explored the mechanism by which lycopene and lutein, two major human plasma carotenoids, are transported.
Methods and results: CD36 was a putative candidate for this uptake, 3T3-L1 cells were treated with sulfosuccinimidyl oleate, a CD36-specific inhibitor. sulfosuccinimidyl oleate-treated cells showed a significant decrease in both lycopene and lutein uptake as compared to control cells. Their uptake was also decreased by partial inhibition of CD36 expression using siRNA, whereas the overexpression of CD36 in Cos-1 cells increased their uptake. Finally, the effect of CD36 on carotenoid uptake was confirmed *ex vivo* in cultures of adipose tissue explants from $CD36^{-/-}$ mice, which exhibited reduced carotenoid uptake as compared to wild-type mice explants.
Conclusion: For the first time, we report the involvement of a transporter, CD36, in carotenoid uptake by adipocytes and adipose tissue.

Keywords:
Adipocytes / Adipose tissue / CD36 / Lutein / Lycopene

1 Introduction

Carotenoids represent a large group of naturally occurring pigments that are found in plants, algae, and various microorganisms. Dietary carotenoids or foods rich in these colorful pigments, are considered to be beneficial in the prevention of several diseases, including certain cancers, cardiovascular diseases, and eye diseases [1]. Recently, we showed that lycopene, one of the main carotenoids found in the Western diet, also impacts adipose tissue biology [2, 3]. Previously, we demonstrated that lycopene was able to modulate the inflammatory response of adipose tissue to the same extent as a high-fat diet or TNF-α treatment [2]. The ability of lycopene to impact adipose tissue was also strongly suggested by the association found between high lycopene intake and low-waist circumferences as well as low-visceral and subcutaneous fat masses as documented by Sluijs *et al.* [4].

Lycopene represents more than half of the total carotenoid concentration found in adipose tissue [5]. Several vehicles including physiological ones, such as LDL or BSA, can deliver lycopene to adipocytes [6]. However, the mechanism of lycopene uptake remains elusive. Notably, the process of carotenoid uptake has been studied in the intestine. In enterocytes, transporters such as scavenger receptor Class B Type I (SR-BI) [7] and cluster of differentiation 36 (CD36) [8] have been involved in lutein, lycopene, and β-carotene uptake.

CD36 is an 88 kDa member of a family of cell surface glycoproteins. As a scavenger receptor, CD36 recognizes a

Correspondence: Dr. Jean-François Landrier, UMR 1260 INRA, Faculté de Médecine, 27 boulevard Jean-Moulin, 13385 Marseille Cedex 5, France
E-mail: jf.landrier@univmed.fr
Fax: +33-4-91-78-21-01

Abbreviations: FBS, fetal bovine serum; **SSO,** sulfosuccinimidyl oleate

broad variety of ligands including β-carotene and fatty acids. In several cell types, such as adipocytes or muscle cells, CD36 facilitates long chain fatty acid transport [9, 10]. Indeed, CD36 null mice present a reduced uptake of fatty acids in adipose tissue as compared to wild-type mice [10, 11], suggesting an active role for CD36 in the uptake of fatty acids by adipose tissue. With respect to enterocytes, the involvement of CD36 is less defined. However, the function of CD36 is dependent not only on its localization along the intestine [12] but also on the type of fatty acids (saturated long very long chain fatty acids *versus* analogs of long chain fatty acids [13, 14]). The mechanism of fatty acid uptake has been partially elucidated by the identification of a specific binding domain in the extracellular domain of CD36 [14]. Recent studies strongly suggest a role for the lingual CD36 as a gustatory lipid sensor [15, 16]. Thus, in addition to directly binding fatty acids, CD36 could also impact sensing or signaling pathways. Because carotenoids share several aspects of their metabolism with lipids and because CD36 has been involved in β-carotene uptake by the intestine [8], we hypothesize that carotenoid uptake by adipose tissue could be mediated at least in part by CD36.

In this study, we explored the role of CD36 in lycopene and lutein uptake by adipocytes. The role of CD36 on lycopene and lutein uptake was also confirmed in cultures of adipose tissue explants from $CD36^{-/-}$ mice.

2 Materials and methods

2.1 Chemicals

(E)-lycopene (≥95% pure), echinenone (≥97% pure), and lutein (≥95% pure) were generous gifts from Dr. Catherine Caris-Veyrat (INRA UMR406, Avignon, France). Sodium taurocholate, pyrogallol, and tetrahydrofuran were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Rabbit polyclonal CD36 antibody was purchased from Santa Cruz Biotechnology (Heidelberg, Germany). DMEM containing 4.5 g/L glucose and trypsin-EDTA (500 and 200 mg/L, respectively) was purchased from BioWhittaker (Fontenay-sous-Bois, France). Fetal bovine serum (FBS) was purchased from Biomedia (Issy-les-Moulineaux, France). Non-essential amino acids, penicillin/streptomycin, optimum, PBS TRIzol reagent, random primers, and Moloney murine leukemia virus reverse transcriptase were obtained from Invitrogen (Carlsbad, CA, USA). SYBR Green reaction buffer was purchased from Eurogentec (Angers, France). JetPEI reagent was purchased from Polyplus Transfection (Illkirch, France). DharmaFECT reagent and siRNA sequences were purchased from Dharmacon RNA Technologies (Epsom, UK). The Protease inhibitor mixture was purchased from Roche Diagnostics (Meylan, France). All solvents used were HPLC grade and obtained from Carlo Erba – SDS (Peypin, France). Human CD36 cDNA was a generous gift from Dr. Johannes von Lintig (Case Western Reserve University, Cleveland, OH, USA).

2.2 Cell culture

3T3-L1 preadipocytes (ATCC, Manassas, VA, USA) were seeded in 3.5-cm diameter dishes at a density of 15×10^4 cells/dish as previously reported. Cells were grown in DMEM supplemented with 10% FBS at 37°C in a 5% CO_2 humidified atmosphere. To induce differentiation, two-day postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 48 h with 0.5 mM isobutylmethylxanthine, 0.25 μmol/L dexamethasone, and 1 μg/mL insulin in DMEM supplemented with 10% FBS [17]. The cultures were then propagated with DMEM supplemented with 10% FBS and 1 μg/mL insulin. All treatments were performed on day eight.

2.3 Adipose tissue explants culture

Adipose tissue explants (epididymal white adipose tissue) were recovered from wild-type ($n = 4$) and $CD36^{-/-}$ mice ($n = 4$), as previously reported [2]. Briefly, after dissection adipose tissue was rinsed in saline buffer, and cut in small explants (approx. 2 mm^3). These explants were randomly placed in DMEM supplemented with 10% FBS at 37°C, in a 5% CO_2 humidified atmosphere. Incubation of explants of adipose tissues with THF-solubilized lycopene or lutein was performed for 3 h. Carotenoid uptake measurement was performed as described below.

2.4 Transfection assays

Cos-1 cells were seeded in 10-cm diameter dishes at a density of 6×10^5 cells/dish, as previously described [18]. Cells were grown in DMEM supplemented with 10% FBS at 37°C in a 5% CO_2 humidified atmosphere. Human CD36 cDNA was subcloned into a pIRES neo3-basic vector with EcoRI. The construct was sequenced by Cogenics (Meylan, France). Cells were transfected overnight using JetPEI reagent at a ratio of 6 μL JetPEI *per* 3 μg plasmid/dish. After an additional 48 h of incubation, uptake assays were performed for 60 min.

3T3-L1 differentiated cells were seeded in 6-well plates and were transfected with either CD36 siRNA or a non-targeting siRNA, following the manufacturer's instructions (Dharmacon, Lafayette, CO, USA). Briefly, cells were transfected overnight using a mixture of 100 nM siRNA and 2 μL of DharmaFECT reagent/well. Next, the media were replaced with complete media. After an additional 48 h of incubation, uptake assays were performed for 60 min.

2.5 Carotenoid uptake measurement

3T3-L1 and Cos-1 cells were incubated with DMEM supplemented with 2 μM of lycopene or lutein solubilized in THF for 1 h at 37°C [6]. At the end of each experiment, media were harvested. Cells were then washed twice with 0.5 mL ice-cold PBS containing 10 mmol/L taurocholate, to eliminate adsorbed lycopene or lutein, scraped, and collected in 0.5 mL of PBS. Carotenoid uptake was estimated as the amount found in the scraped cells. All the samples were stored at −80°C under nitrogen with 0.5% pyrogallol to prevent oxidation during lipid extraction and HPLC analysis. Aliquots of cell samples containing protease inhibitors but not pyrogallol were used to estimate protein concentrations using a bicinchoninic acid kit (Pierce, Montluçon, France). Data are the mean of three independent experiments each performed in triplicate.

For experiments using the explants of adipose tissues, incubation with the physiologically relevant blood concentration of THF-solubilized lycopene (2 μM) [6] or lutein (2 μM) was performed for 3 h. Explants were then rinsed three times with 0.5 mL ice-cold PBS containing 10 mmol/L taurocholate to eliminate adsorbed lycopene or lutein. Carotenoid uptake was estimated as the amount found in the explants. All of the samples were stored at −80°C under nitrogen with 0.5% pyrogallol to prevent oxidation during lipid extraction and HPLC analysis. Aliquots of samples containing protease inhibitors but not pyrogallol were used to estimate protein concentrations using a bicinchoninic acid kit (Pierce).

2.6 Effect of sulfosuccinimidyl oleate (SSO) on carotenoid uptake

3T3-L1 cells were pretreated with either DMSO (control) or SSO, a specific irreversible chemical inhibitor of fatty acid binding to CD36 [19], at 200 μM for 60 min. Next, cells received DMEM supplemented with carotenoids solubilized in THF and SSO at the preincubation concentration for 60 min. The toxic effect of SSO on cells was monitored by LDH quantification. At the end of the incubation, cells were scraped and the absorbed carotenoids were measured as previously described. Data are the mean of three independent experiments each performed in triplicate.

2.7 Real-time quantitative RT-PCR analysis

Total cellular RNA was extracted from cells using TRIzol reagent according to the manufacturer's instructions [20]. The cDNA was synthesized from 1 μg of total RNA using random primers and Moloney murine leukemia virus reverse transcriptase. Real-time quantitative RT-PCR analyses were performed using the Mx3005P Real-time PCR System (Stratagene, La Jolla, CA, USA), with the following primers: for CD36, 5′-CTTGTGTTTTGAACATTTCTGC TT-3′ and 5′-TGTACCTATACTGTGGCTAAATGAGA-3′; for 18S, 5′-CGCCGCTAGAGGTGAAATTCT-3′ and 5′-CAT-TCTTGGCAAATGCTTTCG-3′. For each condition, expression was quantified in duplicate and 18S mRNA was used as the endogenous control in the comparative cycle threshold (C_T) method. Data are the mean of three independent experiments each performed in triplicate.

2.8 Western blot analysis

To verify the expression of CD36, cell pellets were resuspended in 200 μL of NP-40 buffer by mild sonication. Next, 15–30 μg of protein was denatured in sample buffer containing SDS and β-mercaptoethanol, separated on a 10% SDS-PAGE gel, and electroblotted overnight onto PVDF membranes. Non-specific binding sites on the membranes were blocked using PBS with 0.1% Tween 20 (TBS-T) and 5% BSA, followed by incubation with the appropriate antibody diluted in the TBS-T with 5% BSA solution overnight at 4°C. The relative amount of primary antibody was detected after 1 h of incubation at room temperature with species-specific horseradish peroxidase-conjugated secondary antibody diluted in the TBS-T with 5% BSA solution. The peroxidase activity was assessed using ECL Plus (Pierce). Similar procedures were carried out to monitor β-actin expression.

2.9 Carotenoid extraction and HPLC analysis

Carotenoids were extracted from 500 μL of the aqueous samples using the following method. The mixture obtained after the addition of μL of ethanol containing echinenone, which serves as an internal standard, was extracted twice with hexane. The hexane phases obtained after centrifugation (500 g, 5 min, 25 ± 3°C) were pooled and evaporated to dryness under nitrogen, and the dried extract was dissolved in 100 μL of methanol/methyl-*tert* butyl ether/ethyl acetate (50:40:10, v/v/v). A volume of 80 μL was used for HPLC analysis.

Carotenoids were separated using a 250 × 4.6 nm RP C_{30}, 5 μm YMC column (Interchim, Montluçon, France) and a guard column. The mobile phase consisted of 50% methanol, 40% methyl-*tert* butyl ether, and 10% ethyl acetate. The flow rate was 1 mL/min, and the column was kept at a constant temperature (35°C). The HPLC system comprised a Dionex separation module (P680 HPLC Pump and ASI-100 Automated Sample Injector, Dionex SA, Voisins le Bretonneux, France) and a Dionex UVD340U photodiode array detector. Carotenoids (lycopene, lutein, and echinenone) were identified by retention time and spectral analysis (190–500 nm) and were compared to pure (>95%) standards. Lycopene was quantified at 472 nm, while lutein and echinenone were quantified at 450 nm. Quantification was performed using

Chromeleon software (version 6.50 SP4 Build 1000, Dionex) comparing peak areas with standard reference curves.

2.10 Statistical analyses

Data are expressed as the mean ± SEM. Significant differences between control and treated groups were determined by the unpaired Student's *t*-test using Statview software (SAS Institute, Cary, NC, USA). *p*-Values below 0.05 were considered significant.

3 Results

3.1 SSO decreased the uptake of carotenoids in 3T3-L1 adipocytes

In the first set of experiments, we assessed whether CD36 was involved in lycopene and lutein absorption in adipocytes using the 3T3-L1 cells. Figure 1 shows that the uptake of carotenoids in cells treated for 60 min with 200 μM SSO, a chemical inhibitor of CD36, significantly decreased (lycopene and lutein at 2 μM) ($p<0.05$), as compared to the control cells. The percentage of lycopene uptake varied from 7.2% without inhibitor to 3.1% after the treatment with SSO. Lutein uptake ranged from 3% without inhibitor to 1.1% after addition of SSO. There were no observed differences in the rates of inhibition for lutein and lycopene. Furthermore, SSO was not toxic to 3T3-L1 cells (data not shown).

3.2 Inhibition of CD36 expression decreased the uptake of carotenoids in 3T3-L1 adipocytes

Because the treatment of 3T3-L1 cells with SSO decreased carotenoid uptake, we hypothesized that partial inhibition of CD36 expression in these cells should also diminish lycopene and lutein uptake. This hypothesis was verified by transfecting 3T3-L1 cells with a siRNA pool designed against CD36 and a non-targeting siRNA pool (control). Transfection with CD36-specific siRNA resulted in a significant decrease in CD36 mRNA and protein expression (60% inhibition) as compared to control values (Fig. 2A and B). Cells were incubated with 2 μM of each carotenoid for 60 min. As shown in Fig. 2C, inhibition of CD36 expression significantly decreased carotenoid uptake by 3T3-L1 cells. The percentage of lycopene uptake varied from 7.8% in siControl transfected cells to 5.2% in siCD36 transfected

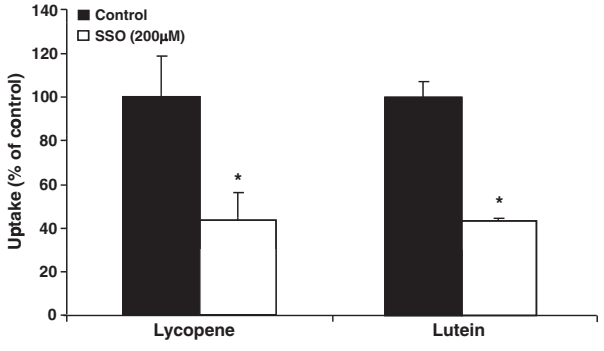

Figure 1. SSO inhibited carotenoid uptake by 3T3-L1 cells. Cells were pretreated with SSO (200 μM for 60 min at 37°C), followed by treatment for 60 min with lycopene or lutein solubilized in THF (2 μM) containing SSO. Cellular carotenoid uptake was measured as described in Section 2. Mean ± SEM representative of three independent experiments; *$p<0.05$.

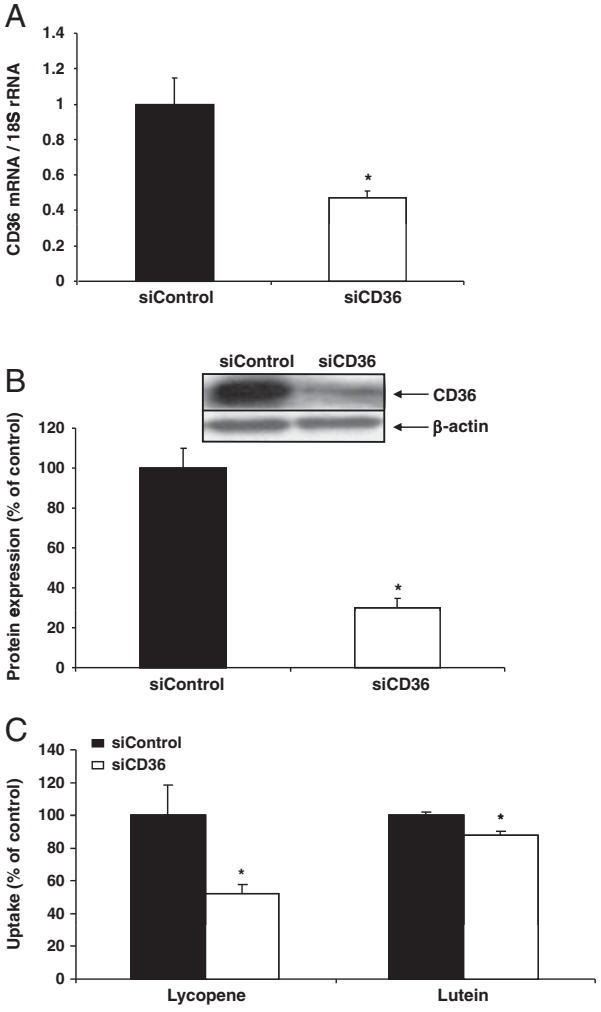

Figure 2. Inhibition of CD36 expression decreased carotenoid uptake by 3T3-L1 cells. 3T3-L1 cells were transfected with either non-targeted siRNA pool (siControl) or with siRNA pool targeted against mouse CD36 (siCD36) for 72 h. (A) qPCR analysis of CD36 mRNA level. Data were normalized to 18S. (B) Immunoblot analysis of CD36 expression in transfected cells. (C) Cells were then incubated with either lycopene or lutein solubilized in THF (2 μM) for 60 min, and the cellular carotenoid uptake was measured as described in Section 2. Mean ± SEM representative of three independent experiments; *$p<0.05$.

cells. Lutein uptake ranged from 3.5% in siControl transfected cells to 3% in siCD36 transfected cells.

3.3 Overexpression of CD36 enhanced the uptake of carotenoids in Cos-1 cells

The results described above strongly suggested that CD36 plays a role in the uptake of carotenoids. Therefore, we hypothesized that CD36 overexpression should enhance carotenoid uptake. To test this hypothesis, we studied Cos-1 cells transiently transfected with human CD36 cDNA. Overexpression of CD36 in Cos-1 cells, that normally have no detectable basal CD36 expression (Fig. 3A), enhanced carotenoid uptake by 30% following a 60-min incubation with 2 μM lycopene or lutein (Fig. 3B). The percentage of lycopene uptake varied from 0.18% in control cells to 0.31% in cells over expressing CD36. Lutein uptake ranged from 2.8% in control cells to 3.5% in CD36 overexpressing cells.

3.4 Adipose tissue explants of CD36$^{-/-}$ mice displayed a reduced uptake of carotenoids

To demonstrate the involvement of CD36 in carotenoid uptake by adipose tissue, we experimented with CD36$^{-/-}$ mice. Explants of adipose tissue from CD36$^{-/-}$ mice or wild-type mice were dissected and cultured *ex vivo* in the presence of lycopene or lutein for 3 h. Compared to explants from wild-type mice, adipose tissue explants from CD36$^{-/-}$ mice displayed a significant reduction in carotenoid uptake (*i.e.* 30 and 33% for lycopene and lutein, respectively, Fig. 4). The percentage of lycopene uptake varied from 2.5% in wild-type mice to 1.67% in CD36$^{-/-}$ mice. Lutein uptake ranged from 0.36% in wild-type mice to 0.21% in CD36$^{-/-}$ mice.

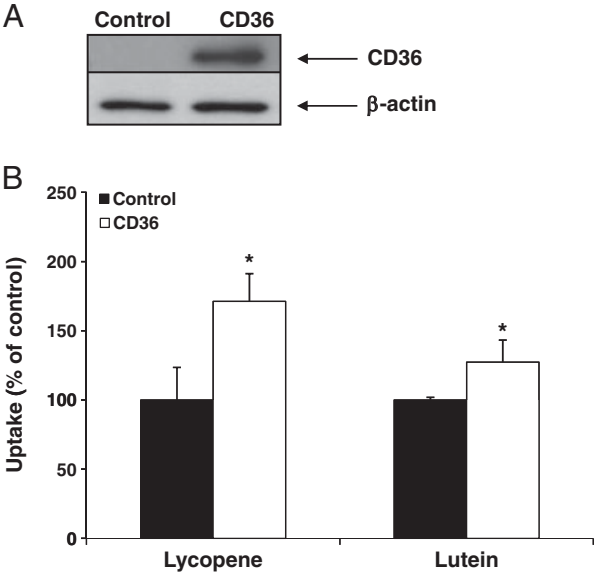

Figure 3. CD36 overexpression increased carotenoid uptake by Cos-1 cells. Cos-1 cells were transiently transfected with either the control plasmid (Control) or with a plasmid encoding human CD36 (CD36) for 72 h. (A) Immunoblot analysis of CD36 expression in transfected cells. (B) Cells were then incubated with either lycopene or lutein solubilized in THF (2 μM) for 60 min, and cellular carotenoid uptake was measured as described in Section 2. Mean ± SEM representative of three independent experiments; $^{*}p < 0.05$.

4 Discussion

Adipose tissue is considered to be the main storage site for carotenoids. This tissue, which is mainly composed of adipocytes, expresses CD36 at a high level [11]. Furthermore, CD36, which is also expressed in the intestine, is involved in β-carotene uptake in this organ. Thus, it was reasonable to evaluate the involvement of this protein in carotenoid uptake. In this study, we showed for the first time that CD36 is involved in the uptake of two major human plasma carotenoids, lycopene, and lutein, by adipose tissue and adipocytes. These two molecules were chosen because they are the main carotene and xanthophyll found in human adipose tissue. Furthermore, data on the involvement of CD36 in β-carotene uptake have already been published [8]. Notably, the concentration of lycopene and lutein chosen for this study (2 μM for both carotenoids) was within the blood concentrations achievable by carotenoid supplementation [21–23].

In a first set of experiments, we used a 3T3-L1 cellular model (murine adipocytes) in which both lycopene and lutein uptake was impaired by SSO, a chemical inhibitor that binds specifically to CD36 [19, 24]. Similarly, a partial

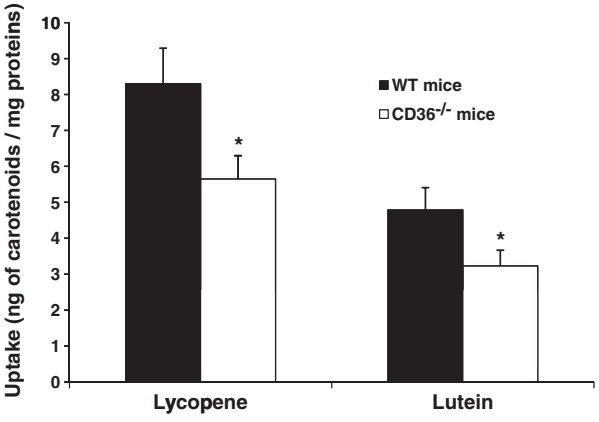

Figure 4. Adipose tissue explants from CD36 knockout mice had reduced carotenoid uptake. Explants of adipose tissue from wild-type ($n = 4$) and CD36$^{-/-}$ mice ($n = 4$) were incubated for 3 h in culture medium supplemented with lycopene (2 μM) or lutein (2 μM). Carotenoid uptake was measured as described in Section 2. Data were normalized to protein quantity. Mean ± SEM; $^{*}p < 0.05$.

reduction of CD36 expression by RNA interference reduced the intracellular accumulation of lycopene and lutein in adipocytes.

In parallel experiments, we decided to use a cellular model that does not express CD36 to evaluate the impact of CD36 overexpression on carotenoid uptake. Cos-1 cells, which do not normally express CD36, were transfected. After the transfection, Cos-1 cells increased their carotenoid uptake by 30%. These results are in agreement with those reported by Van Bennekum *et al*. [8] who showed that the overexpression of CD36 in Cos-7 cells significantly increased β-carotene uptake. Interestingly, these data strongly support a role for CD36 in the uptake of carotenoids.

Because adipose tissue is composed of approximately 80% adipocytes and because carotenoids are highly lipophilic molecules, we hypothesized that the effect observed on carotenoid uptake by adipocytes could be reproduced in adipose tissue. Thus, we demonstrated that carotenoid uptake in adipose tissue was, at least in part, CD36-dependent. To further investigate this hypothesis, we used a methodology based on the *ex vivo* culture of explants of adipose tissue [2]. In these experiments, we dissected the adipose tissue of wild-type mice and $CD36^{-/-}$ mice. This methodology is particularly relevant since it bypasses the low bioavailability of lycopene in mice [25]. As expected, explants dissected from adipose tissue of $CD36^{-/-}$ mice displayed reduced carotenoid uptake as compared to adipose tissue explants from wild-type mice.

Altogether our data argue for a role of CD36 in the uptake of carotenoids by adipocytes and adipose tissue.

Our data raise a question regarding the mechanism by which CD36 mediates these effects. In the intestine, CD36 has been suggested to act as a mixed micelle docking protein that contains a specific fatty acid binding domain [14]. Here we used THF as a vehicle to deliver carotenoids to the cells [6]. With such a vehicle, micelle structures are not shaped to deliver carotenoids to cells and, therefore, we cannot rule out the possibility that carotenoids bind directly to CD36, which promotes their transfer to the membrane [14, 26]. It is also possible that carotenoids bound to albumin or other proteins that were present in the culture medium, resulting in a carotenoid–protein complex capable of binding to CD36. Alternatively, the SSO-induced chemical inhibition of CD36 and the impaired expression of CD36 (partially *in vitro*, *via* RNA interference, or totally *in vivo via* knockout) could also have impaired the CD36-mediated cell sensing or signaling [9]. Such a modulation of cell signaling or sensing could have resulted in the expression or activity of other transporters putatively implicated in carotenoids uptake. In support of this, the inhibition of CD36 did not fully impair carotenoid uptake by adipocytes in our conditions, which strongly suggests that other mechanisms are involved in carotenoid uptake by adipocytes and adipose tissue. This process may involve either a passive diffusion mechanism or other membrane transporters. This last assumption would require further experiments to be tested.

In summary, the data presented here provide new information on the mechanisms involved in lycopene and lutein uptake by adipose tissue. Indeed, for the first time we showed that two of the main human plasma carotenoids are taken up by adipocytes and adipose tissue *via* a facilitated process that involves CD36. These findings open new areas of research dedicated to the involvement of transporters in the cellular uptake of carotenoids.

This work was supported by grants from the community's sixth framework program (LYCOCARD, integrated European project), INRA, and INSERM.

The authors have declared no conflict of interest.

5 References

[1] Krinsky, N. I., Johnson, E. J. Carotenoid actions and their relation to health and disease. *Mol. Aspects Med.* 2005, *26*, 459–516.

[2] Gouranton, E., Thabuis, C., Malezet, C., El Yazidi, C. *et al.*, Lycopene inhibits proinflammatory cytokine and chemokine expression in adipose tissue. *J. Nutr. Biochem*, 2010, in press, Doi: 10.1016/j.jnotbio.2010.04.016.

[3] Tourniaire, F., Gouranton, E., von Lintig, J., Keijer, J. *et al.*, beta-Carotene conversion products and their effects on adipose tissue. *Genes Nutr.* 2009, *4*, 179–187.

[4] Sluijs, I., Beulens, J. W., Grobbee, D. E., van der Schouw, Y. T. Dietary carotenoid intake is associated with lower prevalence of metabolic syndrome in middle-aged and elderly men. *J. Nutr.* 2009, *139*, 987–992.

[5] Chung, H. Y., Ferreira, A. L., Epstein, S., Paiva, S. A. *et al.*, Site-specific concentrations of carotenoids in adipose tissue: relations with dietary and serum carotenoid concentrations in healthy adults. *Am. J. Clin. Nutr.* 2009, *90*, 533–539.

[6] Gouranton, E., Yazidi, C. E., Cardinault, N., Amiot, M. J. *et al.*, Purified low-density lipoprotein and bovine serum albumin efficiency to internalise lycopene into adipocytes. *Food Chem. Toxicol.* 2008, *46*, 3832–3836.

[7] Reboul, E., Abou, L., Mikail, C., Ghiringhelli, O. *et al.*, Lutein transport by Caco-2 TC-7 cells occurs partly by a facilitated process involving the scavenger receptor class B type I (SR-BI). *Biochem. J.* 2005, *387*, 455–461.

[8] van Bennekum, A., Werder, M., Thuahnai, S. T., Han, C. H. *et al.*, Class B scavenger receptor-mediated intestinal absorption of dietary beta-carotene and cholesterol. *Biochemistry* 2005, *44*, 4517–4525.

[9] Silverstein, R. L., Febbraio, M. CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. *Sci. Signal.* 2009, *2*, re3.

[10] Coburn, C. T., Knapp, F. F., Jr., Febbraio, M., Beets, A. L. *et al.*, Defective uptake and utilization of long chain fatty acids in muscle and adipose tissues of CD36 knockout mice. *J. Biol. Chem.* 2000, *275*, 32523–32529.

[11] Febbraio, M., Abumrad, N. A., Hajjar, D. P., Sharma, K. *et al.*, A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J. Biol. Chem.* 1999, *274*, 19055–19062.

[12] Nassir, F., Wilson, B., Han, X., Gross, R. W., Abumrad, N. A. CD36 is important for fatty acid and cholesterol uptake by the proximal but not distal intestine. *J. Biol. Chem.* 2007, *282*, 19493–19501.

[13] Drover, V. A, Ajmal, M., Nassir, F., Davidson, N. O. *et al.*, CD36 deficiency impairs intestinal lipid secretion and clearance of chylomicrons from the blood. *J. Clin. Invest.* 2005, *115*, 1290–1297.

[14] Drover, V. A., Nguyen, D. V., Bastie, C. C., Darlington, Y. F. *et al.*, CD36 mediates both cellular uptake of very long chain fatty acids and their intestinal absorption in mice. *J. Biol. Chem.* 2008, *283*, 13108–13115.

[15] Laugerette, F., Passilly-Degrace, P., Patris, B., Niot, I. *et al.*, CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. *J. Clin. Invest.* 2005, *115*, 3177–3184.

[16] Niot, I., Poirier, H., Tran, T. T., Besnard, P. Intestinal absorption of long-chain fatty acids: evidence and uncertainties. *Prog. Lipid Res.* 2009, *48*, 101–115.

[17] Landrier, J. F., Gouranton, E., Yazidi, C. E., Malezet, C. *et al.*, Adiponectin expression Is induced by vitamin E *via* a peroxisome proliferator-activated receptor -dependent mechanism. *Endocrinology* 2009;

[18] Hassan, M., El Yazidi, C., Landrier, J. F. *et al.*, Phloretin enhances adipocyte differentiation and adiponectin expression in 3T3-L1 cells. *Biochem. Biophys. Res. Commun.* 2007, *361*, 208–213.

[19] Harmon, C. M., Abumrad, N. A. Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. *J. Membr. Biol.* 1993, *133*, 43–49.

[20] Landrier, J. F., Malezet-Desmoulins, C., Reboul, E., Marie Lorec, A. *et al.*, Comparison of different vehicles to study the effect of tocopherols on gene expression in intestinal cells. *Free Radic. Res.* 2008, *42*, 523–530.

[21] Allen, C. M., Schwartz, S. J., Craft, N. E., Giovannucci, E. L. *et al.*, Changes in plasma and oral mucosal lycopene isomer concentrations in healthy adults consuming standard servings of processed tomato products. *Nutr. Cancer* 2003, *47*, 48–56.

[22] Olmedilla, B., Granado, F., Southon, S., Wright, A. J. *et al.*, Serum concentrations of carotenoids and vitamins A, E, and C in control subjects from five European countries. *Br. J. Nutr.* 2001, *85*, 227–238.

[23] Thurmann, P. A., Schalch, W., Aebischer, J. C., Tenter, U., Cohn, W. Plasma kinetics of lutein, zeaxanthin, and 3-dehydro-lutein after multiple oral doses of a lutein supplement. *Am. J. Clin. Nutr.* 2005, *82*, 88–97.

[24] Harmon, C. M., Luce, P., Beth, A. H., Abumrad, N. A. Labeling of adipocyte membranes by sulfo-*N*-succinimidyl derivatives of long-chain fatty acids: inhibition of fatty acid transport. *J. Membr. Biol.* 1991, *121*, 261–268.

[25] Moussa, M., Landrier, J. F., Reboul, E., Ghiringhelli, O. *et al.*, Lycopene absorption in human intestinal cells and in mice involves scavenger receptor class B type I but not Niemann-Pick C1-like 1. *J. Nutr.* 2008, *138*, 1432–1436.

[26] Baillie, A. G., Coburn, C. T., Abumrad, N. A. Reversible binding of long-chain fatty acids to purified FAT, the adipose CD36 homolog. *J. Membr. Biol.* 1996, *153*, 75–81.

