Unsaturated fatty acids promote bioaccessibility and transepithelial transport of carotenoids and α-tocopherol by Caco-2 cells

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Unsaturated fatty acids promote bioaccessibility and basolateral secretion of carotenoids and α-tocopherol by Caco-2 cells

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Abstract

Bioavailability of carotenoids and tocopherols from foods is determined by the efficiency of transfer from food/meal to mixed micelles during digestion, incorporation into chylomicrons for trans-epithelial transport to lymphatic/blood system, and distribution to target tissues. Fats and oils are important factors for facilitating the absorption of lipophilic compounds. However, dietary fats and oils are composed of various types of saturated and unsaturated fatty acids which may differentially impact the bioavailability of carotenoids and tocopherols from foods. We have investigated the effects of several common commercial lipids on bioavailability using an in vitro digestion model and Caco-2 human intestinal cells. Meals consisted of mixed salad vegetables containing a single test lipid. Micellarization and cellular uptake of β-carotene (βC) and lycopene (LYC) during small intestinal digestion was increased by lipids rich in unsaturated fatty acids: soybean oil > olive > canola > butter. In contrast, type of lipid minimally affected the bioaccessibility of lutein (LUT) and zeaxanthin (ZEA). To examine the influence of type of dietary triglyceride on uptake and basolateral secretion of carotenoids, Caco-2 cells grown on Transwell membranes were incubated with micellar mixtures of fatty acids (1.0 mM) mimicking the types and ratio of saturated to unsaturated (mono- + poly-unsaturated) fatty acids (FA) present in butter (70 : 30), olive oil (7 : 93) and soybean oil (11 : 89). Cells were exposed to micelles containing βC, LUT, α-tocopherol (α-TC) and a mixture of test fatty acids. Uptake and basolateral secretion of βC, LUT and α-TC were greater in cells pre-treated with mixtures enriched in unsaturated compared to saturated FA and these effects were mediated by increased assembly and secretion of chylomicrons. These results suggest that dietary fats/oils rich in unsaturated fatty acids promote carotenoid and α-TC bioavailability by enhancing their micellarization during digestion and intestinal transport.

Introduction

Delivery of carotenoids, their metabolites and fat soluble vitamins to tissues is required for modulating their cellular activities. Absorption of such compounds parallels that of other dietary lipophiles and drugs. This involves sequential solubilization in lipid droplets, transfer to bile salt micelles containing lipid digestion products, delivery to the brush border membrane of small intestinal epithelial cells for uptake, possible cleavage to retinal and other metabolites, incorporation into chylomicrons and other lipoproteins for secretion into lymph, and distribution to target tissues.[1-3] The extent to which lipophilic micronutrients and phytochemicals in plant foods are absorbed is dependent on multiple factors that can affect one or more of these processes. Such factors include chemical structures of carotenoids and vitamins, food matrix, type and extent of food processing and preparation, and composition of the meal, as well as physiologic status and genetics of the individual.[2-4] For example, co-consumption of dietary fat is essential for absorption of lipid soluble micronutrients and phytochemicals.[2,3,5] However, there is a lack of clarity about how much and what types of fat optimally enhance the absorption of carotenoids and fat soluble vitamins. For example, addition of 5g groundnut oil to spinach curry improved plasma retinol to normal in malnourished children.[6] Other studies have shown that $\geq$ 12g fat provided maximum absorption of carotenoids from unprocessed western-style vegetable salad by healthy adults.[7-9] In contrast, absorption of carotenes and free LUT ingested in carotenoid rich spreads appeared to be similar when consumed with either 3 or 36g fat, although absorption of LUT ingested as LUT esters was enhanced only when the meal contained high fat.[10] Studies with rats and human volunteers have consistently shown that dietary triglycerides (TG) rich in mono-saturated fatty acids have a greater tendency to enhance carotenoid absorption than TG rich in saturated and poly-unsaturated fatty acids,[9, 11, 12] although

the outcome can be affected by food matrix.[13] Similarly, digestion products of dietary lipids differentially affect both the bioaccessibility (i.e., micellarization; [2,3,14,15]) and absorption[13, 16-19] of carotenoids and α-TC.

The primary objective of the studies reported below was to systematically investigate the influence of type of dietary fat on individual processes associated with the digestion and absorption of meals rich in carotenoids and α-TC. Either no fat, butter, olive oil, canola oil or soybean oil was added to a western-style salad that had been tested previously in a bioavailability trial with healthy human subjects.[9] Salad was subjected to simulated gastric and small intestinal digestion to determine the effects of each dietary lipid source on the efficiency of micellarization and uptake by differentiated cultures of Caco-2 cells. Also, monolayers of Caco-2 cells on membrane inserts were used to examine the effects of micelles containing mixtures of fatty acids resembling those in the butter, olive oil and soybean oil on apical to basolateral transport of LUT, βC and α-TC, and the relationship with the secretion of TG and apo-lipoprotein B (apoB).

Results

Effect of type of dietary TAG on bioaccessibility of carotenoids and α-TC

Digestive stability

Mean recovery of total carotenoids after simulated gastric and small intestinal digestion of salad puree containing 3.0% fat was 83.8 ± 8% (LUT, 80.2%; ZEA, 87.5%; α-carotene (AC), 93.3%; βC, 71.6%; LYC, 86.2%). The ratio of all-*trans* to *cis* isomers of LUT, βC and LYC was not significantly different in salad and chyme. ZEA esters were partially hydrolyzed during digestion with the relative amounts of all-*trans* ZEA, ZEA mono-esters and ZEA di-esters accounting for

22.3%, 5.0% and 72.7%, respectively, in chyme. Recovery of carotenoids and α-TC, the ratio of all-*trans* to *cis* isomers of carotenoids, and hydrolysis of ZEA esters were not significantly affected by type or amount of fat added to salad before digestion (data not shown). Addition of 3% (wt/wt) butter, olive oil, canola oil and soybean oil to salad puree increased α-TC content from 10.9 μg/g salad to 11.6, 15.2, 16.1 and 14.5 μg/g salad, respectively. Recovery of α-TC after completion of the small intestinal phase of digestion of salad puree with the different fats was 91.8-95.2% and not significantly affected by exogenous source of fat.

Micellarization of carotenoids and α-TC during simulated digestion

The efficiency of transfer of carotenoids from salad puree containing either 0% or 3.0% fat to the micelle fraction of chyme was dependent on carotenoid speciation (LUT > non-esterified ZEA > βC > AC > LYC, ZEA esters), the absence or presence of fat, and the source of fat (Table 1). Partitioning of *cis* isomers of LYC in the micelle fraction of chyme was significantly greater than that of all-*trans* isomers. Also, the relative extent of transfer of non-esterified ZEA significantly exceeded that of ZEA esters. Transfer of all carotenoids to the aqueous fraction of chyme was dependent on the generation of mixed micelles as deletion of bile extract during the small intestinal phase of digestion decreased the amount of carotenoids and α-TC in the aqueous fraction to ≤ 2.0 ± 0.2%. The amounts of non-esterified and esterified ZEA, AC, βC and LYC were significantly less in the micelle fraction of chyme generated during digestion of salad containing butter instead of one of the oils. Also, micellarization of AC and βC, but not ZEA orLYC, was significantly greater when olive or soybean oil was substituted for canola oil in salad puree. Transfer of α-TC to micelles during simulated digestion of salad puree with 3% fat ranged from 42-45% and was independent of source of exogenous fat (Table 1).

The extent of micellarization of the carotenoids generally was proportional to the amount of soybean oil (0-8%) added to salad puree when concentrations of digestive enzymes and bile extract remained constant (Fig. 1). This was most evident for the increased amounts of ZEA, carotenes, LYC and α-TC in the aqueous fraction when soybean oil content in salad puree increased from 0% to 1%, and for carotenes, LYC and α-TC when content increased from 3% to 8% (Fig. 1). Micellarization of LUT increased to 53 ± 2% of that in salad puree when soybean oil content was 3%, but surprisingly decreased (35 ± 1%) to less than that of AC and βC when oil content of the salad was increased to 8%.

The accessibility of all *trans* LUT, ZEA, AC, βC nd LYC in micelles generated during small intestinal digestion was confirmed by monitoring their accumulation by monolayers of Caco-2 cells adhered to plastic dishes (Fig. 2). Cellular content of *cis* isomers after 4h incubation with diluted micelle fraction was below the limit of detection. The percentage of all five all-trans carotenoids in apical medium taken up by Caco-2 cells ranged from 7-11% (data not shown). Cell content of all-*trans* LYC was markedly less than that of the other carotenoids regardless of the type and amount of fat added to salad puree (Fig. 2). Uptake of all-*trans* LYC was affected by source of fat as its accumulation from micelles generated during digestion of salad with butter was only 20% that from micelles generated during digestion of salad containing the three oils. Although the concentrations of LUT, ZEA and AC in salad puree were similar (13-16.5 μg/g) and less than that of βC and LYC (23.5 and 23.2 μg/g, respectively), cells accumulated greater quantities of the xanthophylls compared to the more abundant βC and LYC from digested salad regardless of type of fat (Fig. 2**)**. Incubation of cells with micelle fraction of digested meals containing exogenous fat also increased cellular content of α-TC (Fig. 2). Cellular α-TC content after incubation with diluted micelle fraction after digestion of the salad meals containing oils

was twice that in cells incubated with diluted micellefraction generated during digestion of salad with butter.

Effects of micelle fatty acyl composition on basolateral secretion of LUT, βC and α-TC

The concentrations of carotenoids and α-TC in micelles generated during simulated digestion of salad were too low for accurate determination of the influence of different mixtures of fatty acids in the tested dietary fats on their basolateral secretion. Therefore, medium with micelles containing mixtures of fatty acids resembling those generated during digestion of the four dietary fats were prepared (Table 2) to examine the effect of source of dietary fat on uptake and basolateral secretion of intracellular carotenoids. LUT and βC were selected as representative xanthophyll and hydrocarbon carotenoids, respectively. These carotenoids and α-TC were solubilized in Tween 40 for addition to apical medium. The preparations were combined to deliver 2 μmol mixtures of fatty acids and 20 nmol βC, LUT and α-TC to the apical compartment of monolayers grown on inserts. Using this co-exposure strategy, the total amount of the carotenoids and α-TC taken up by cells, *i.e.*, quantities present within cells and in the basolateral compartment, was minimally affected by the presence or sources of fatty acids in the mixed micelles (Fig. 3A). As expected, the amounts Fig. 3B) and secretion efficiency (Fig. 3c) of carotenoids and α-TC into the basolateral compartment were significantly lower in cultures exposed to micelles lacking free fatty acids compared to micelles containing the various mixtures of fatty acids. However, the amounts of LUT, βC and α-TC secreted into the basolateral compartment during the 8h incubation were 177-302% greater when mixtures rich in unsaturated fatty acids were substituted for the mixture rich in saturated fatty acids (Fig. 3B). This was due to more than a two efficiency with which these compounds were secreted from the cell into the

basolateral compartment. The influence of the mixtures rich in unsaturated fatty acids on the transport of LUT, βC and α-TC varied. Secretion of LUT was slightly, but significantly ($p<0.05$), greater in cultures incubated with micelles containing fatty acids resembling those in soybean oil compared to olive and canola oils. In contrast, secretion of βC was greater ($p<0.01$) in cultures exposed to mixtures of fatty acids resembling those in olive oil compared to canola and soybean oils. It should be noted that secretion of α-TCS into the basolateral compartment in cultures lacking exogenous fatty acids was greater than that for LUT and βC. Secretion of of α-TC into the basolateral compartment also exceeded that of LUT and βC when cells were incubated with micelles containing fatty acids resembling each of the dietary fats.

We also examined the effect of chronic pre-exposure of Caco-2 monolayers to micelles containing the different mixtures of fatty acids on secretion of carotenoids and α-TC into the basolateral compartment as routine consumption of a single source of dietary fat with meals is common in developing countries.[20] Medium containing micelles with one of the mixtures of fatty acids (1 mmol final concentration) was added to the apical compartment for 4h per day beginning on day 17 post-confluency and continuing through day 20 post-confluency. On day 21, apical medium containing micelles with the same fatty acyl mixtures as that on days 17-20 and Tween micelles containing LUT, βC and α-TC was added to cultures. Surprisingly, the amounts of LUT, βC and α-TC taken up from the apical compartment by cells chronically incubated with the mixed micelles were approximately 50% that by cells only exposed to such micelles on the day of the experiment (Fig. 4A, no fat bar and 3A). However, both the amounts and the efficiency with which intracellular LUT, βC and α-TC were secreted into the basolateral compartment by cells chronically exposed to the micelles with fatty acid profiles resembling that

of the dietary fats was approximately twice that after a single co-exposure to micelles with the same mixtures of fatty acids (Fig. 4B and 4C compared to 3B and 3C). Secretion of LUT, βC and α-TC into the basolateral compartment by cells chronically exposed to the mixtures of fatty acids also was proportional to the concentration of fatty acids in apical medium (0.5-2.0 mmol/L) for all tested mixtures of fatty acids and significantly greater for mixtures rich in unsaturated fatty acids compared to saturated fatty acids (Table 3).

We examined the possibility that differences in apical uptake by cells chronically and acutely exposed to micelles with the various fatty acids might be associated with alteration of the fatty acyl composition of phospholipids in the plasma membrane. Chronic exposure (17-21d post-confluency) to micelles containing mixtures of fatty acids altered the fatty acyl profile of brush border membrane phospholipids of Caco-2 cells. Daily apical exposure of cells to micelles with fatty acyl mixture resembling olive, canola and soybean oils increased the ratio of unsaturated to saturated fatty acids in brush border membrane by 75% ($p<0.0001$), 9% ($p<0.01$) and 32% ($p<0.0001$), respectively. Chronic exposure to micelles containing fatty acids resembling the composition of butter slightly, but significantly ($p<0.01$), decreased the ratio of unsaturated to saturated fatty acids in brush border membranes by 7%. In contrast, the ratio of unsaturated to saturated fatty acids in the basolateral membrane was not significantly different in control cells (1.0 : 0.93; $p = 0.69$) and cells chronically exposed to micelles with all four mixtures of fatty acids (1.0 : 0.85-0.97; $p = 0.49$).

Micellar mixtures rich in unsaturated fatty acids promote basolateral secretion of LUT, βC and α-TC by enhancing secretion of chylomicrons

The distribution of the carotenoids and α-TC in basolateral medium was determined for cultures treated with mixtures of fatty acids resembling soybean oil. Chylomicrons contained 90 ± 5% of βC and 85 ± 4% of LUT in the basolateral compartment. Residual βC was located in the VLDL fraction, whereas 9 ± 1 and 6 ± 1% of total LUT were present in the VLDL and more dense fractions, respectively. Distribution of α-TC in basolateral medium differed from the carotenoids with the chylomicron fraction, VLDL fraction and the more dense fraction containing 60 ± 2%, 10 ± 1% and 30 ± 1%, of the total, respectively. TG and apoB content in cells and the TG-rich fraction (d<1.006 g/mL) of basolateral medium were measured to assess the differential effects of micelle fatty acyl composition on the trans-epithelial transport of carotenoids and α-TC. TG content in untreated cells and in basolateral medium at 21d post-confluency were 51 and 2.8 μg/well, respectively. Acute exposure to micelles rich in unsaturated fatty acids resembling dietary oils (2 μmol/well) increased TG content in cells by 50-100% and in the TG-rich fraction of basolateral medium 6-10 fold (Fig. 5A**)**. Single exposure of cells to micelles with fatty acids resembling those in butter did not significantly alter TG content in the TG-rich fraction of basolateral medium, despite increasing intracellular TG by 50%. ApoB content in the basolateral medium of control cultures was 455 ng/well. Single exposure to micelles containing the mixtures of fatty acids resembling the dietary oils increased apoB in the TG-rich fraction of basolateral medium 1.2-1.4 fold, whereas apoB content in the basolateral compartment after acute exposure to micelles containing fatty acids resembling butter was only 13% greater than the basal amount (Fig. 5B).

Compared to single exposure to micelles with the mixtures of fatty acids, chronic exposure of cells to micelles rich in saturated fatty acids resembling butter and dietary oils further increased

intracellular TG content (1.9 fold and 3.3-4.1 fold, respectively), and basolateral TG content 2.4 fold and approximately 20-fold, respectively (Fig. 5A). Similarly, apoB in basolateral medium of cells chronically exposed to micelles with fatty acids resembling butter and the oils increased 1.9 and 3.95-4.5 fold, respectively (Fig. 5B). The ratio of TG to apoB in the basolateral medium was markedly greater in cultures acutely exposed to micelles with mixtures of fatty acids resembling the dietary oils compared to the mixture resembling butter (Fig. 5C). The increased amounts of apoB and greater ratio of secreted TG/apoB indicates that the mixtures of fatty acids resembling the dietary oils promoted secretion of a greater number of larger lipoprotein particles. Chronic exposure to the fatty acyl mixtures resembling those of olive and soybean, but not canola, oils further increased the ratio of secreted TG to apoB. The amounts of TG and apoB in the TG-rich fraction of basolateral medium by cells chronically exposed to the mixtures of fatty acids were highly correlated (≥ 0.95) with the secretion of LUT, βC, and α-TC.

Discussion

Co-consumption of fat is known to promote the absorption of health-promoting lipophilic compounds.[2,3] However, the sources and amounts of fat that should be co-consumed for maximal absorption of carotenoids, fat soluble vitamins and other health promoting lipophilic micronutrients and phytochemicals in fruits and vegetables remain unclear. The reported observations represent a systematic *in vitro* examination of the effect of various sources and amounts of fatty acids on the transfer of carotenoids and α-TC in a salad to mixed micelles during simulated digestion, their delivery to differentiated cultures of Caco-2 cells, and the basolateral secretion of these compounds across the monolayer. α-TC in the test meal originated from both the components of the salad itself and the exogenous fats.

The static method used for gastric and small intestinal digestion provides data that aligns well with use of the more complex dynamic digestion models[21,22] and is well correlated with results for bioavailability in human participants.[23] Micellarization during digestion was largely dependent on the presence of exogenous fat and the relative hydrophilicity of the carotenoids (LUT > AC, βC > LYC, ZEA esters) as reported previously.[14,24-26] Xanthophylls, and likely α-TC, are located on the surface of lipid droplets and thus more readily transferred to mixed micelles than hydrocarbon carotenoids that are localized within the core.[27] The efficiency of micellarization of ZEA was markedly below that of lutein likely due to the fact that the majority of the xanthophyll present in wolfberry exists as a di-ester,[28,29] and the ability of the mixed micelles to accommodate the carotenoid esters is less than that of the free xanthophylls.[29] Hydrolysis of carotenoid esters is mediated by carboxyl ester lipase, a relatively abundant enzyme in exocrine pancreatic secretion[30] and its activity in pancreatin utilized in the current study appeared to be insufficient to generate free ZEA for greater incorporation into micelles. Transfer of carotenoids, but not α-TC, from the salad to micelles was significantly greater when the fat source was rich in unsaturated fatty acids (i.e., olive, canola and soybean oils) compared to saturated fatty acids (i.e, butter). This is similar to recent observations involving in vitro digestion of spinach and mixed salad with TG containing unsaturated long chain fatty acids.[15,31] In contrast, Gleize *et al*.[32] recently reported that xanthophylls were better micellarized during digestion of a meal (minced beef with 5% fat and potatoes) with either butter or palm oil compared to the meal containing olive or fish oils. The basis for the different effects of the various fats on micellarization of the xanthophylls is unknown, but may be related to the influence of type of dietary fat on the physicochemical characteristics of generated mixed micelles, food matrix, and the presence of other lipophilic compounds in both the foods and the

exogenous fats.[14,32-34] Unlike carotenoids, micellarization of α-TC was independent of type of fat added to the salad meal. The greater hydrophilicity of the vitamin compared to the C40 carotenoids[35] likely facilitates its partitioning from the oil droplet to bile salt mixed micelles during the small intestinal phase of digestion.

With the exception of LUT, there was a concentration dependent increase in the micellarization of the carotenoids and α-TC for salad containing 0-8% soybean oil. The decreased efficiency of micellarization of LUT in salad containing 8% in raw vegetable mixtures also has been reported to decrease with relatively high concentrations of oils and this may be due to incomplete digestion of the oil in the in vitro system.[14,15] The general influence of amount of exogenous fat on the bioaccessibility of the carotenoids and α-TC is similar to reports that meals containing >12g exogenous fats enhanced the absorption of these compounds compared to meals with ≤8g fat.[7-9, 36] In contrast, Roodenburg *et al*.[10] reported that absorption of LUT esters, but not βC or α-TC, added to a meal as a supplement was enhanced when intake of fat was 36g rather than 3g. This is likely due to the increased secretion of pancreatic enzymes and thus carboxyl ester lipase, as well as bile salts with the high fat load.

After transfer of carotenoids and α-TC from the food matrix to mixed micelles, the compounds are transported across the brush border membrane of absorptive epithelial cells at least in part by various proteins including SR-B1 and NPC1L1.[2,3,37,38] The potential influence of different lipid digestion products on the interaction of micelles with the cell surface has received limited attention. We observed that the percentage of LUT, ZEA, AC, βC. LYC and α-TC in micelles generated during simulated digestion taken up by Caco-2 cells was minimally influenced by the source of fat added to vegetable salad. For example,, uptake of LYC and α-TC was lower when

butter was substituted for one of the vegetable oils in the salad. Gleize *et al*.[32] have reported that uptake of xanthophylls by Caco-2 cells from digested sweetcorn was greater when the meal contained palm and olive oils compared to sunflower and fish oils and that the difference was not correlated with size of the mixed micelles. Various lipid digestion products including acylglycerols, fatty acids, phospholipids and lyso-phospholipids have been shown to differentially affect the uptake of carotenoids from micelles by Caco-2 cells.[14,15,19,39] These data suggest that the sources of dietary lipids may influence apical uptake of micellarized compounds by absorptive epithelial cells.

The strategy for investigating the potential effects of various dietary fats on the secretion of carotenoids across the basolateral membrane of Caco-2 cells integrated several approaches used successfully by other investigators. Mixtures of fatty acids resembling those most abundant in the fat sources of interest were introduced to apical medium in mixed micelles to stimulate secretion of apoB containing TG-rich lipoproteins by Cao-2 cells.[40-42] Lutein and βC served as representative oxy- and hydro-carbon carotenoids. Similar to During *et al*.[23] these carotenoids and α-TC were incorporated into Tween 40 micelles for apical delivery to the cells with the assumption that similar amounts of these compounds would be taken up by cells. Acute exposure of the cells to medium containing the micelles with the various mixtures of fatty acids markedly increased transport of the carotenoids and α-TC into the basolateral compartment, while minimally affecting uptake from apical medium. Micelles containing mixtures of fatty acids resembling the oils rich in unsaturated fatty acids stimulated secretion of the lipophiles to a greater extent than micelles with the mixture of fatty acids resembling butter. This stimulatory effect was correlated with the amounts of TG and apoB secreted into the basolateral compartment. Mono-unsaturated fatty acids and particularly oleic acid are potent inducers of

chylomicron assembly and secretion by Caco-2 cells.[43,44] Similarly, consumption of a single meal with either olive oil or sunflower oil induced a greater lipemic response than the meal with butter.[45] The carotenoids were largely (≥ 85%) located in the TG-rich fractions in basolateral medium, whereas only 60% of secreted α-TC was present in this fraction. It has been shown that absorption of α-TC is mediated by both chylomicron-dependent and chylomicron-independent pathways with the former predominant in the postprandial state.[46]

Cells also were exposed to micelles with the mixtures of fatty acids for 4d before co-exposing to both the complex micelles, carotenoids and α-TG. Daily pre-exposure was limited to 4h because chronic exposure to micelles rich in saturated fatty acids can adversely affect the integrity of the brush border membrane and tight junctions.[40.41,47] Our treatment protocol did not compromise barrier integrity, but altered the fatty acyl composition of the brush border membrane. The decreased uptake of carotenoids and α-TC by cells chronically exposed to the mixtures of fatty acids may have been due to changes in permeability of the brush border membrane and perhaps the activity of specific membrane transporters. In contrast, the amounts and efficiency of secretion of the carotenoids and α-TC across the basolateral membrane were greater than that in response to acute exposure. These increases was correlated with the secretion of greater quantities of TG and apoB. The amounts of carotenoids and α-TC secreted into the basolateral compartment also was proportional to the concentrations of the fatty acids present in the apical compartment. Because chylomicrons and VLDL contain a single molecule of apoB,[48] the increased secretion of apoB and greater ratio of TG to apoB in the TG-rich fraction in the basolateral compartment suggest that the increased basolateral secretion of LUT, βC and α-TC resulted from secretion of a greater number of larger chylomicrons across the basolateral

membrane. The results also suggest that carotenoids and α-TC are not necessarily shunted into intracellular TG rich droplets upon entering the cell, but are more likely to be transferred to the maturing lipoprotein particles in the ER and Golgi.[1]

Results of the current study demonstrate that co-consumed dietary lipid, in the form of fats and oils, may modulate carotenoid and vitamin E bioavailability at multiple points in the digestive and absorptive process. While type and amount of lipid will impact micellarization and intestinal uptake, these effects are modest compared to apparent impact of lipid type and quantity on chylomicron synthesis and secretion. The effects of the amounts and types of mixtures of fatty acids on the micellarization, uptake and trans-epithelial transport by Caco-2 cells align well with our recent finding that the absorption of carotenoids from a mixed salad meal with 20g of exogenous fat exceeded that from the salad containing either 3 g or 8g and that there was a tendency for carotenoids to be more efficiently absorbed when the meal contained fats rich in unsaturated fatty acids (i.e., canola and soybean oils) compared to butter.[9] Combined these results suggest that co-consumption of modest amounts of fats/oils rich in unsaturated fatty acids, particularly mono-unsaturated fatty acids, may promote bioavailability of fat soluble micronutrients and phytochemicals from commonly consumed vegetables.

Experimental

Reagents

Dulbecco's minimal essential medium (DMEM), antibiotics, enzymes, porcine bile extract and bile acids were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum, trypsin, L-glutamine and non-essential amino acids were purchased from Invitrogen (Chicago, IL). Lipid-reduced FBS was purchased from Hyclone (Logan, Utah). Fatty acids were obtained

from Matreya Inc. (Pleasant Gap, PA). HPLC grade solvents and other reagents were purchased from Fisher Scientific (Pittsburgh, PA).

Salad meal

A typical western salad was prepared with fresh tomatoes (90g), carrots (62g), spinach (50g), romaine lettuce (25g), and dried wolfberry (*Lycium barbarum*; 7g). The composition of this test salad was selected to match that of a previous clinical trial [9] in order to facilitate comparison of in vivo and in vitro responses. Sun-dried wolfberries were purchased from an Asian market in Columbus, OH, and were re-hydrated with an equal volume of deionized water for at least 1 h before preparing a puree. Other vegetables and tomatoes were purchased from local markets, washed with tap water, rinsed with deionized water, drained and homogenized with kitchen blender to a puree after adding wolfberry puree. The salad puree contained the following quantities of carotenoids: LUT (89% all-*trans*), 15.2 ± 0.5 μg/g; zeaxanthin (ZEA; 95% di-esters; 3% mono-esters, 2% non-esterified), 12.8 ± 0.7 μg/g; all-*trans* α-carotene (AC), 16.5 ± 0.3 μg/g; β-carotene (βC; 94% all-*trans)*, 23.5 ± 1.0 μg/g; and, lycopene (LYC; 95% all-*trans*), 23.2 ± 0.8 μg/g. Endogenous α-TC content was 10.9 ± 0.2 μg/g. Salad puree was stored in aliquots at -80°C under N_2 and used for *in vitro* digestion within four weeks. There was no significant change in carotenoid and α-TC content during storage.

Effect of type of TG on bioaccessibility of carotenoids and α-TC

Either 3% (wt/wt) butter, olive oil, canola oil or soybean oil was added to salad puree (2.4 g) to determine the effects of lipid source on stability and efficiency of micellarization of carotenoids and α-TC during simulated gastric and small intestinal digestion. Butter was melted in microwave for 20 sec before addition to reaction tubes at 37°C. In a separate experiment, the

amount of soybean oil added to salad puree was varied (1%, 3% or 8% wt/wt) to determine the dose-dependency of micellarization of the carotenoids and α-TC. Simulated gastric and small intestinal digestion was performed as previously reported[49], except that simulated small intestinal reactions contained 2.8, 1.4 and 4.8 mg/mL of acetone-precipitated porcine pancreatin (Sigma product P4251), porcine pancreatic lipase, and porcine bile extract, respectively. After completion of the small intestinal phase of digestion, an aliquot of chyme was centrifuged at 12,000 x g, 4°C, for 45 min to separate the aqueous fraction from undigested materials. Supernatant was passed through a syringe filter (0.2 μmeter pores) to collect the mixed micelle fraction. Aliquots of chyme and aqueous fraction were stored at -20°C under nitrogen gas for a maximum of one week before extraction and analysis of carotenoids and α-TC. Recovery as determined by dividing the concentration of total carotenoids and α-TC in chyme by the concentrations in pre-digested salad puree was 83.8 ± 8% and 93 ± 1.4%, respectively. Bioaccessibility was determined by dividing the concentrations of carotenoids and α-TC in the filtered aqueous fraction (subsequently referred to as the micelle fraction) by that in pre-digested pureed salad meal with exogenous fats.

Caco-2 cell cultures

Caco-2 human cells (HTB37) were purchased from the American Type Culture Collection (ATCC, Rockville MD). This parental cell line does not express β-carotene oxygenase activity and therefore does not cleave carotenoids to retinaldehyde and other apo-carotenoids.[50] Maintenance of the cells has been described previously[49] and differentiated cultures were used for experiments between passages 26 and 32. For experiments, cells were seeded in either 6-well dishes (2.5 x 10^5 cells/well) or onto polycarbonate inserts (2.5 x 10^5 cells per Transwell®, 3.0

μm pore size; Millipore, MA). Fresh medium was added to cultures every second day and the day prior to initiating experiments.

Effect of source of TG on uptake of carotenoids and α-TC by Caco-2 cells

Aliquots of the micelle fraction generated during digestion of the salad were diluted (1:4) with serum-free DMEM and added to wells with monolayers of Caco-2 cells adhered to the dish surface at 12-14 d post-confluency. After 4 h incubation (37˚C, 90% humidity, 95% air: 5% CO_2), medium was removed and the monolayer washed once with ice cold HEPES (15 mmol/L) buffered saline containing 2 g/L bovine serum albumin, pH 7.0, and twice with cold HEPES buffered saline without albumin. Possible adverse effects of 4h exposure of the monolayers to diluted micelle fraction generated during digestion of vegetable salad containing different sources and amounts of fat/oils were considered. Phase contrast microscopic observation of the monolayer and individual cells and the amount of protein per well of control and treated cultures were not significantly different. Cells were collected and stored under N_2 at -80˚C for a maximum of one week prior to analysis of carotenoid and α-TC content.

Effect of fatty acyl composition of dietary TG on basolateral secretion of LUT, βC and α-TC

Three different experiments were performed to determine the influence of mixtures of fatty acids resembling the fatty acyl composition of butter, olive oil, canola oil and soybean oil on apical uptake and basolateral secretion of LUT, βC and α-TC. In the first experiment, medium containing both micelles with a mixture of fatty acids mimicking one of fats added to salad and Tween micelles with the carotenoids and α-TC was added to the apical compartment of

monolayers grown on membrane inserts at 21 post-confluency. Cultures of this age were used as Levy *et al*.[51] reported that maximum chylomicron assembly and secretion occurred 3-4 weeks after monolayers became confluent. A second experiment with 21 d post-confluent cultures determined the effect of the concentration (0-2 mmol/L) of the fatty acid mixture mimicking that of soybean oil to determine the impact of fatty acyl dose on the basolateral secretion of the carotenoids and α-TC. As individuals are likely to habitually ingest a particular source of dietary triglycerides and fatty acyl composition of membrane phospholipids reflects dietary intake, cultures of Caco-2 cells were exposed to apical medium containing micelles with the different fatty acid mixtures (but without carotenoids and α-TC) for 4 h daily on days 17-20 post-confluency. Washed monolayers were exposed to fresh medium with the same fatty acid mixture and Tween micelles containing LUT, βC and α-TC on day 21.

Media containing mixed micelles with the various mixtures of fatty acids for addition to the apical compartment were prepared as described elsewhere.[49,52] The initial composition of the medium was fetal bovine serum free DMEM supplemented with 2 mmol/L L-glutamine, 1% non-essential amino acids, 0.5 mmol/L phenol red, antibiotics and 1 mmol sodium taurocholate, This was added to dried preparations of a lipophilic mixture to prepare test medium to prepare test medium containing 0-2 mmol/L mixture of fatty acids mimicking the fatty acyl composition of test TG (Table 2), 0.5 mmol/L mono-olein, 0.2 mmol/L phosphatidylcholine (PC,; 0.2 mmol/L lyso-PC and 0.05 mmol/L cholesterol,. LUT (10 umol/L), βC (10 umol/L) and α-TC (10 umol/L) were delivered in Tween 40 micelles (0.1% final concentration) as described by During *et al*.[53] Medium (2 mL) containing the micelles with the fatty acyl profile mimicking the dietary lipid of interest and 10 μmol/L LUT, βC and α-TC was added to apical compartment of washed monolayers. Phenol red-free DMEM (3 mL) containing 1% fetal bovine serum, 2 mmol/L L-

glutamine, 1% non-essential amino acids and antibiotics was added to the basolateral compartment. For all three experiments, apical and basolateral media were collected after 8h and monolayers were rinsed once with cold PBS containing 2g bovine albumin/L and twice with cold PBS. Phenol red flux to basolateral membrane and protein content per insert were not significantly altered by any of the indicated treatments. Cells were removed from surface of the insert by scrapping with a plastic scraper, collected in PBS, and pelleted by centrifugation (400 x g, 4°C, 5 min). Aliquots of media and the cell pellet were stored under N_2 at -80˚C for a maximum of one week before analysis. Stability of carotenoids and α-TC in medium after 8h incubation in the absence of cells was 95 ± 2% and 96 ± 1%, respectively. Phenol red transport from the apical to the basolateral chamber and cell protein content per well were measured to assess possible adverse effects of the treatments on the monolayer There were no significant differences in these markers for control cultures and cultures treated once or chronically (5x) with the micelles with any of the fatty acid mixtures. Uptake refers to the total quantity of the carotenoids and α-TC in the cell monolayer and the basolateral compartment. Basolateral secretion refers to the quantities of carotenoids and α-TC transported into basolateral medium.

Analyses of carotenoids and α-TC

Thawed samples (1-3mL) of salad puree, chyme and micelle fraction were extracted into 3 vol tetrahydrofuran : hexane (1:1, v/v) containing 0.1% triethylamine. Extraction was repeated three times and organic phases were combined and dried at room temperature under nitrogen. The film was resolubilized in methanol (MeOH): methyl-tert-butyl-ether (MTBE) : (1:1) and analyzed immediately. Caco-2 cell pellets were thawed before adding 2 mL PBS and 1.5 mL ethanol containing 34.6 mmol/L sodium dodecyl sulfate and 4.5 mmol/L butylated hydroxytoluene. The suspension was sonicated for 20 sec and carotenoids were extracted as

above. Carotenoids and α-TC in apical and basolateral media also were extracted as above. The HPLC system consisted of a Waters 2695 separation module with a 2996 photodiode array detector controlled by an Empower workstation (Waters, Milford, MA). An analytical polymeric YMC^{TM} C_{30} column (4.6 mm i.d. x 150 mm, particle size 5 μm; Waters, Milford, MA) protected by C_{18} guard column (4.6 mm i.d. x 50 mm) with the same packing was used to separate the carotenoids and α-TC. Sample and column temperatures were maintained at 10°C and 35°C, respectively. Carotenoids and α-TC were eluted from the column using a gradient described by Weller and Breithaupt.[54] Solvent A consisted of MeOH : MTBE: 2% ammonium acetate (83:15:2, v/v/v) and solvent B consisted of methanol : MTBE : water (8:90:2, v/v/v). The following linear gradient was used at flow rate 0.6 mL/min : 0 to 10 min, 100% A; 10 to 40 min, 50% A; 40 to 50 min, 100% B; 50 to 55 min, 100% A. Elution of carotenoids and α-TC were monitored at 450 nm and 292 nm, respectively. Carotenoids and α-TC were identified by comparison of retention time and spectra with pure standards. Concentrations were determined by comparison of area under the curve with those generated by analysis of six different concentrations of pure (>98%) all-*trans*-carotenoids and α-TC. The extinction coefficients ($E^{1\%}$1cm) used were 2550 for LUT at 445 nm, 2540 for ZEA at 450 nm, 2710 and 2592 for all-trans AC and all-trans βC at 450 nm, and 3450 for all-*trans* LYC at 470 nm.[55,56] The extinction coefficient used for α-TC was 75.8 at 292 nm.[57] *Cis*-isomers of carotenoids were also identified by comparison of retention time and spectra, and concentrations were quantitatively estimated using extinction coefficients for their respective all *trans*-isomers. Echinenone was used as an internal standard with recovery ranging from 93 to 96%. Detection limit was identified as a signal to noise ratio ≥ 3 and was ≥ 5 ng/mL for carotenoids and ≥ 20 ng/mL α-TC.

TG, apoB, carotenoid and α-TC in cells and basolateral medium

Initially**,** basolateral medium was subjected to sequential density gradient ultracentrifugation as described by Luchoomun and Hussain[58] to characterize the distribution of carotenoids and α-TC in large chylomicrons [Svedberg units (Sf) >400], small chylomicrons (Sf = 60-400), VLDL particles (Sf = 20-60, 1.5 mL) and the remaining fraction (referred to as "other"). Fractions rich in large and small chylomicrons were pooled for accurate quantification of carotenoids and α-TC. In subsequent experiments basolateral medium was centrifuged (28,000 x g for 18h at 4°C) to collect the TG-rich fraction (d.< 1.019 g/mL)[59] to measure TG, apoB, carotenoids and α-TC. Cell pellets were homogenized on ice in PBS containing a cocktail of protease inhibitors. Lipids in homogenized cells were extracted into 4 vol chloroform-methanol (2:1, v/v). Dried samples were re-solubilized in tert-butanol: MeOH : Triton X-100 (3:1:1, v/v/v) before vortexing and centrifuging (34,000 x g, 120 sec). The supernatant was collected for TG and apoB content. Intracellular and secreted TG was determined with Sigma kit TR0100 according to manufacturer's procedure to distinguish free glycerol from glycerol generated during hydrolysis of TAG by lipoprotein lipase. Caco-2 cells synthesize both apoB-48 and apoB-100 unlike enterocytes that synthesize apoB-48 only.[60] Intracellular and secreted apoB were measured by ELISA using AssayMax Human apoB kit containing polyclonal antibodies that recognizes both apoB-48 and apoB-100 (AssayPro, St. Charles, MO). Results are reported as total μg apoB based on the apoB standard provided in the kit.

Plasma membrane fatty acyl profile

Fatty acyl composition of membrane phospholipids reflects dietary intake of esterified fatty acids.[61] Therefore, cultures of Caco-2 cells were incubated in apical medium without carotenoids and α-TC, but containing micelles with fatty acyl mixtures that mimic those in soybean oil (0-2

mmol/L total fatty acids) for 4h daily from 17-20d post-confluency. Then medium in the apical and basolateral compartments was aspirated and replaced by complete DMEM containing 7.5% FBS until the next day. At 21d post-confluency, fresh medium with micelles containing mixtures of fatty acids was added to apical compartment and medium with 1% FBS to the basolateral compartment as above. After 8h, monolayers were washed. Brush border and basolateral membranes were isolated from Caco-2 cells according to Ellis *et al*.[62] The specific activity of sucrase[63] and Na+-K+-ATPase[64] in the brush border and basolateral membrane vesicles were 14-fold and 17-fold greater, respectively, than in homogenate. Fatty acyl composition of membrane phospholipids was determined according to Miwa.[65] Briefly, fatty acyl hydrazides were prepared after saponification and extracted into hexane. Fatty acyl hydrazides were separated on a J'sphere ODS-M 80 column (250 x 4.6 mm i.d., particle size 4μm, Waters, Milford, MA) with a guard cartridge (J'sphere ODS-M 80) using isocratic elution with acetonitrile : methanol : water (75:11:14) maintained at pH 4-5 at flow rate 1.2 mL/min. Elution was monitored at 400 nm and specific fatty acid derivatives were identified by comparing retention time, spectral characteristics and area under curve with fatty acid standards.

Miscellaneous

Protein content of cell homogenates was quantified using the bicinchoninic acid assay kit with bovine serum albumin as standard (Pierce, Rockford, IL). Monolayer integrity of cells on inserts was assessed by apical to basolateral flux of phenol red.[66] Apical to basolateral flux of phenol red in cultures containing 0 (control) to 2 mmol/L fatty acids in the apical compartment, i.e., 0.052 ± 0.002% of phenol red present in the apical chamber at beginning of experiment was transported to the basolateral chamber during the 8h incubation period." **Statistics**

Data are expressed as means ± standard deviation (SD) and were analyzed for statistically significant differences using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The effect of different TAG on recovery, efficiency of micellarization of carotenoids and α-TC, and their uptake by Caco-2 cell were analyzed using one way ANOVA followed by Bonferroni and Duncan's Multiple Range Tests. Effects of different types of lipid mixtures and acute vs. chronic exposure to such mixtures on quantities and amounts of TAG and apoB in cells and in basolateral medium were similarly analyzed. A two-way ANOVA was performed to identify differences in transport of carotenoids and α-TC in cultures incubated with the various types and amounts (0-2 mmol/L) of fatty acyl mixtures followed by Tukey's HSD (honestly significant difference) test. Statistically significant differences were set at $p< 0.05$. Pearson's correlation coefficients (r) were calculated to determine associations between carotenoids, α-TC and TAG, apoB and ratio of TAG/apoB. All variables were checked for normal and homogeneous variance using Levene's test. Data were log-transformed for analysis when variance lacked homogeneity.

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Table 1. Dietary oils (3% wt:wt) promote partitioning of carotenoids, , but not α-TC, in mixed micelles during simulated digestion of salad to greater extent than butter. *

Carotenoids	Efficiency of micellarization (%)				
	No fat	Butter	Olive oil	Canola oil	Soybean oil
Total LUT	10.3 ± 0.3%	46.1 ± 1.1[b]	49.2 ± 2.4[a]	52.0 ± 1.4[a]	52.5 ± 1.8[a]
trans-LUT	10.3 ± 0.3%	45.7 ± 1.1[b]	48.5 ± 2.4[a]	52.1 ± 1.4[a]	52.4 ± 2.1[a]
cis-Lut	nd	50.0 ± 1.2[a]	54.3 ± 1.2[a]	50.9 ± 1.1[a]	53.4 ± 1.2[a]
Total ZEA	5.3 ± 0.5%	13.1 ± 2.1[b]	17.5 ± 4.2[a]	17.7 ± 1.3[a]	18.6 ± 5.5[a]
Free-ZEA	5.3 ± 0.5%	46.4 ± 1.7[b]	55.2 ± 1.2[a]	55.9 ± 1.3[a]	56.9 ± 1.2[a]
ZEA mono-esters	nd	5.1 ± 0.6[b]	8.7 ± 0.5[a]	9.1 ± 0.6[a]	8.2 ± 1.1[a]
ZEA di-esters	nd	6.3 ± 0.8[b]	9.5 ± 1.0[a]	11.0 ± 1.0[a]	10.5 ± 1.5[a]
AC	1.4 ± 0.3%	7.2 ± 0.3[d]	14.8 ± 0.9[b]	12.8 ± 0.4[c]	16.9 ± 1.7[a]
Total βC	nd	9.2 ± 0.5[c]	17.7 ± 1.6[a]	14.9 ± 0.6[b]	20.2 ± 1.4[a]
trans-βC		9.3 ± 0.7[c]	18.1 ± 1.4[a]	15.0 ± 1.0[b]	20.5 ± 1.2[a]
cis-βC		8.5 ± 0.5[c]	12.7 ± 0.5[b]	13.3 ± 0.4[b]	5.4 ± 0.8[a]
Total LYC	nd	1.6 ± 0.3[b]	5.4 ± 0.8[a]	5.5 ± 0.6[a]	5.1 ± 0.6[a]
trans-LYC		1.5 ± 0.3[c]	5.1 ± 0.7[a]	5.3 ± 0.5[a]	4.6 ± 0.4[b]
cis-LYC		2.7 ± 0.3[b]	8.2 ± 1.5[a]	7.5 ± 1.8[a]	10.5 ± 2.3[a]
α-TC	8.2 ± 0.5%	42.0 ± 3.9[a]	45.2 ± 3.0[a]	45.1 ± 4.2[a]	41.8 ± 5.2[a]

*Efficiency of micellarization is the (concentration of carotenoids and α-TC in the micelle fraction after digestion of cassava with indicated fat divided by the concentrations in the pre-digested salad meal) x 100%. Data are means ± SD for n=4 independent simulated digestions of salad containing 3% test fat. nd, not detected. Means without a common letter in a row differ significantly ($p < 0.01$).

Table 2. Fatty acyl mixtures in micelles prepared to resemble the fatty acyl profile of fats added to salad puree.*

Fatty acids	Butter mimic	Olive oil mimic	Canola oil mimic	Soybean oil mimic
Caprylic acid, C8:0	5.8			
Capric acid, C10:0	3.0			
Lauric acid, C12:0	5.2			
Myristic acid, C14:0	17.0			
Palmitic acid, C:16:0	35.1	11.9	4.9	12.4
Stearic acid, C18:0	12.2	0	2.2	4.3
Oleic acid, C18:1	21.7	78.1	68.2	25.7
Linoleic acid,C18:2	0	10.0	24.7	57.6
SFA: MUFA: PUFA	78:22:0	12:78:10	7:68:25	17:26:58

Data from US Department of Health and Human Services and US Department of Agriculture 2005 Dietary Guidelines for Americans were used to prepare mixtures of fatty acids that mimic those most abundant in each commerical fat. .Palmitoic (16:1) and linolenic (18:3) acids were not included in mixtures. Last row indicates the ratio of saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA) and poly-unsaturated fatty acids (PUFA) present in each mixture.

Table 3. Secretion of LUT, βC and α-TC (pmoL/well) to basolateral compartment in cultures of Caco-2 cells chronically exposed to micelles containing fatty acids resembling the composition of indicated dietary fats is proportional to fatty acyl content in apical medium and types of fatty acids.[1, 2]

Type and amount of fatty acyl mixture	β-carotene	Lutein	α-tocopherol
Butter mimic			
0.5mM	71 ± 17^{hC}	180 ± 35^{gB}	503 ± 31^{hA}
1.0mM	108 ± 25^{gC}	299 ± 52^{fB}	709 ± 34^{fA}
2.0mM	237 ± 39^{fC}	422 ± 59^{eB}	961 ± 51^{eA}
Olive oil mimic			
0.5mM	449 ± 36^{dB}	406 ± 42^{eB}	732 ± 36^{fA}
1.0mM	904 ± 71^{bB}	634 ± 59^{cC}	1180 ± 96^{dA}
2.0mM	1281 ± 56^{aB}	864 ± 35^{aC}	1716 ± 93^{aA}
Canola oil mimic			
0.5mM	312 ± 37^{eB}	301 ± 34^{fB}	687 ± 75^{fA}
1.0mM	643 ± 47^{cB}	512 ± 57^{dC}	1018 ± 94^{eA}
2.0mM	941 ± 83^{bB}	717 ± 46^{bC}	1533 ± 101^{bA}
Soybean oil mimic			
0.5mM	248 ± 52^{fC}	316 ± 22^{fB}	572 ± 49^{gA}
1.0mM	463 ± 53^{dB}	402 ± 40^{eC}	957 ± 91^{eA}
2.0mM	680 ± 74^{cB}	537 ± 53^{dC}	1327 ± 111^{cA}

[1] Data are mean ± SD, n = 6 independent treatments per group.

[2] Different lower case letters as superscripts within column indicate significant differences, $p<0.05$, as determined by two-way ANOVA followed by Tukey's HSD (honestly significant difference) test. Different upper case letters as superscripts within row indicate significant differences, $p<0.05$, as determined by two-way ANOVA followed by Tukey's HSD test.

Figure Legends

Fig. 1. Extent of partitioning of ZEA, AC, βC LYC and α-TC in mixed micelles during simulated digestion is proportional to amount of soybean oil added to salad. Means include all *trans* and *cis* isomers of LUT, βC and LYC, as well as non-esterified and esterified ZEA. Data are means ± SD for four independent digestions. The presence of a different letter above error bars indicates that source of dietary fat significantly ($p < 0.01$) affected different extent of micellarization of the carotenoid. nd = not detected.

Fig. 2. Addition of vegetable oils (3% wt:wt) to salad results in greater intracellular levels of AC, βC, LYC and α-TC compared to that in digested salad with butter. Data are means ± SD for 6 independent exposures of cells. Presence of a different letter above bars indicates significant differences ($p<0.05$) of fat source for each carotenoid.

Fig. 3. Uptake and basolateral secretion of LUT, βC and α-TC by Caco-2 cells co-exposed to micelles containing different mixtures of fatty acids, carotenoids and α-TC. *Panel A*. Uptake (amount in cells and basolateral compartment) after co-exposure to mixed micelles with 2 μmol fatty acids mimicking those in dietary sources of fats and Tween micelles containing 20 nmol LUT, βC and α-TC for 8h. A replicate set of cells (control) were exposed to Tween micelles containing LUT, βC and α-TC, but no fatty acids (data not shown). *Panel B*. Quantities of LUT, βC and α-TC secreted into basolateral compartment. *Panel C*. Secretion efficiency. This represents the percentage of LUT, βC and α-TC taken up by the cells that was secreted into the basolateral compartment. Data are means ± SD for 6 cultures per treatment. Significant differences are indicated by different letters above the bars.

Fig. 4. Effect of chronic exposure of Caco-2 cells to different mixtures of fatty acids on uptake and basolateral secretion of LUT, βC and α-TC. Cultures were exposed to medium with mixed micelles containing the indicated fatty acyl mimics for 4h daily on days 17-20 post-confluency. At 21d, cultures were co-exposed to the micelles with the same mixtures of fatty acids and Tween micelles containing 20 nmol LUT, βC and α-TC as in Figure 3. *Panel A*. Uptake. *Panel B*. Quantities of compounds secreted into the basolateral compartment. Panel C. Secretion efficiency. This is the percentage of each compound taken up by the cell that was secreted into basolateral compartment. . Data are means ± SD for 6 cultures per treatment. Significant differences are indicated by the presence of different letters above the bars.

Fig. 5. Acute and chronic exposure of cultures to micelles rich in unsaturated fatty acids significantly increases secretion of TG and apoB into basolateral compartment.

Fig. 1

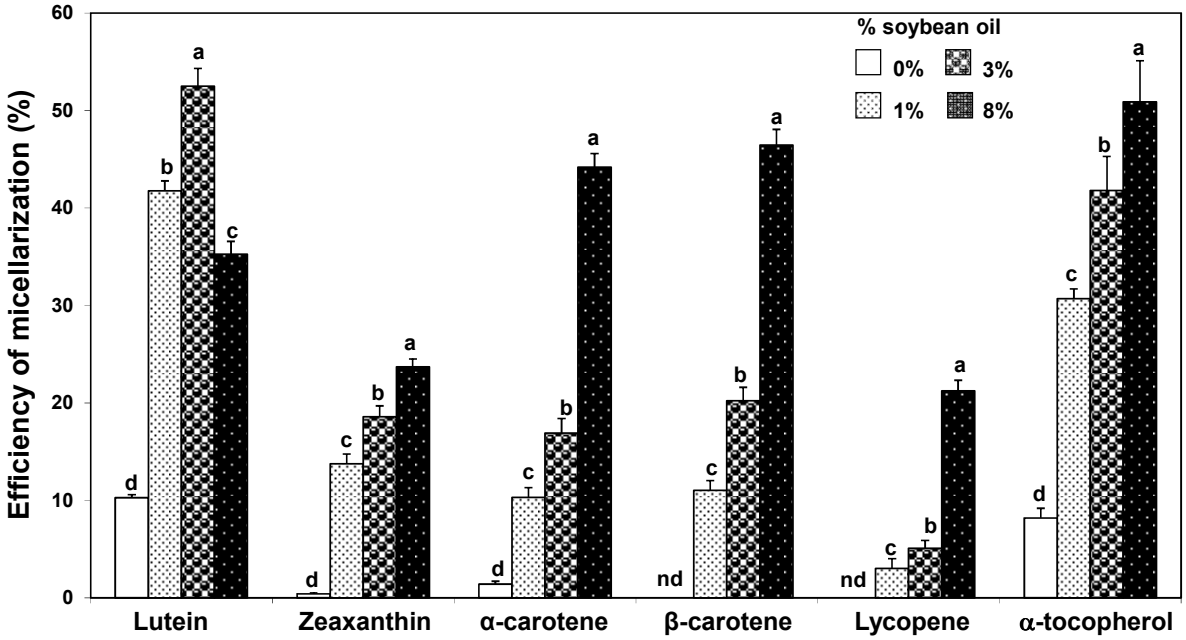

Efficiency of micellarization (%)
60
50
40
30
20
10
0
% soybean oil
0%
3%
1%
8%
a
b
c
d
nd
Lutein
Zeaxanthin
α-carotene
β-carotene
Lycopene
α-tocopherol

Fig. 2

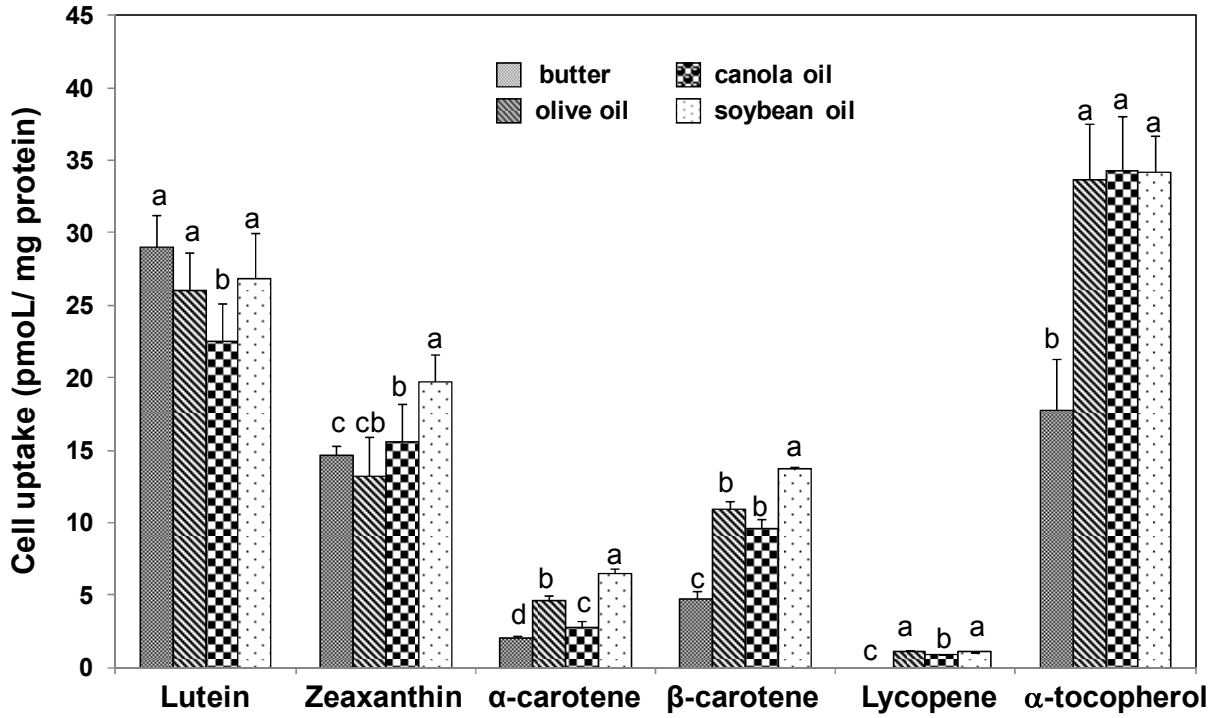

Cell uptake (pmoL/ mg protein)
45
40
35
30
25
20
15
10
5
0
butter
olive oil
canola oil
soybean oil
a a b a
c cb b a
d b c a
c b b a
c a b a
b a a a
Lutein
Zeaxanthin
α-carotene
β-carotene
Lycopene
α-tocopherol

Fig. 3

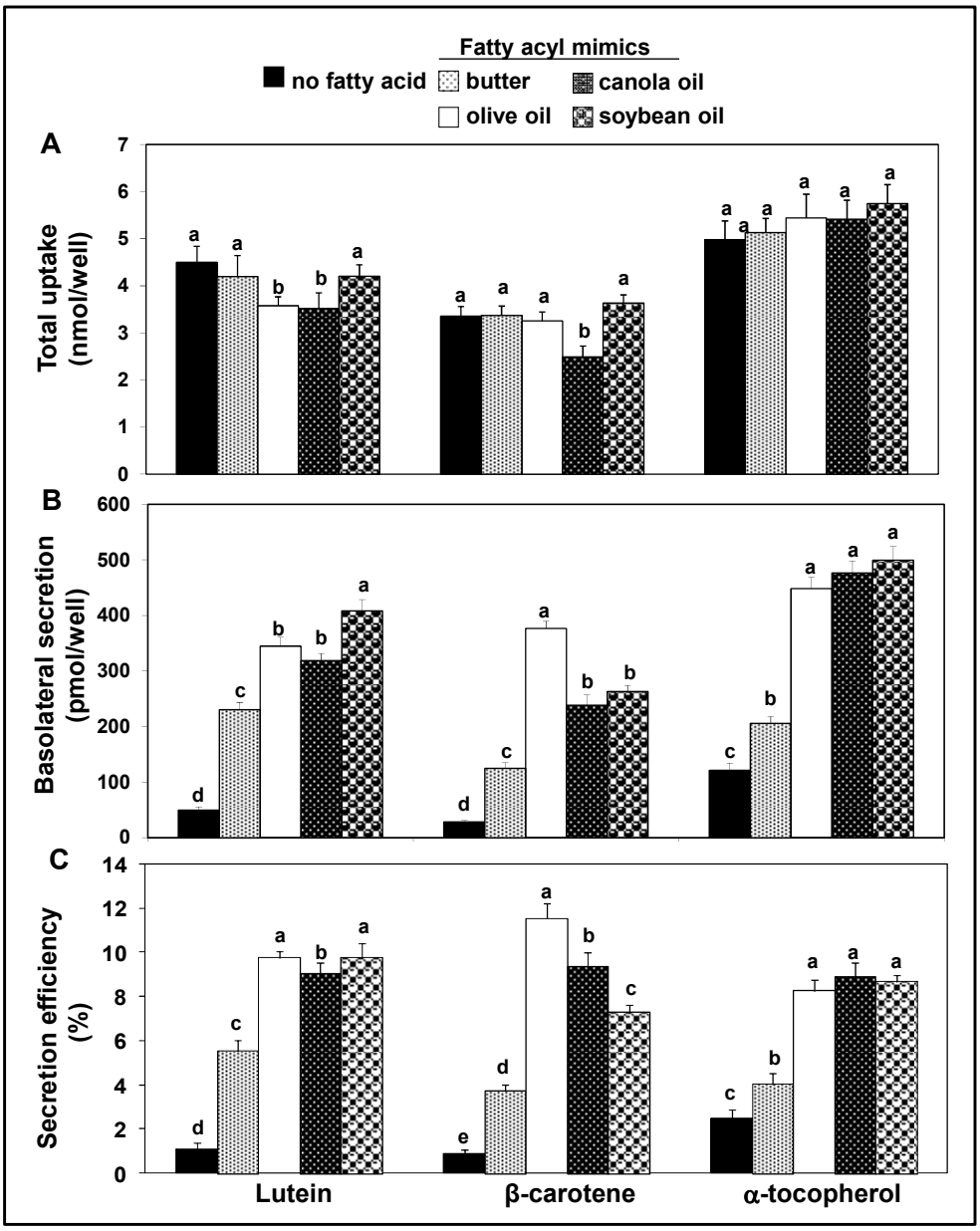

Fatty acyl mimics
no fatty acid
butter
canola oil
olive oil
soybean oil
A
Total uptake (nmol/well)
B
Basolateral secretion (pmol/well)
C
Secretion efficiency (%)
Lutein
β-carotene
α-tocopherol

Fig. 4

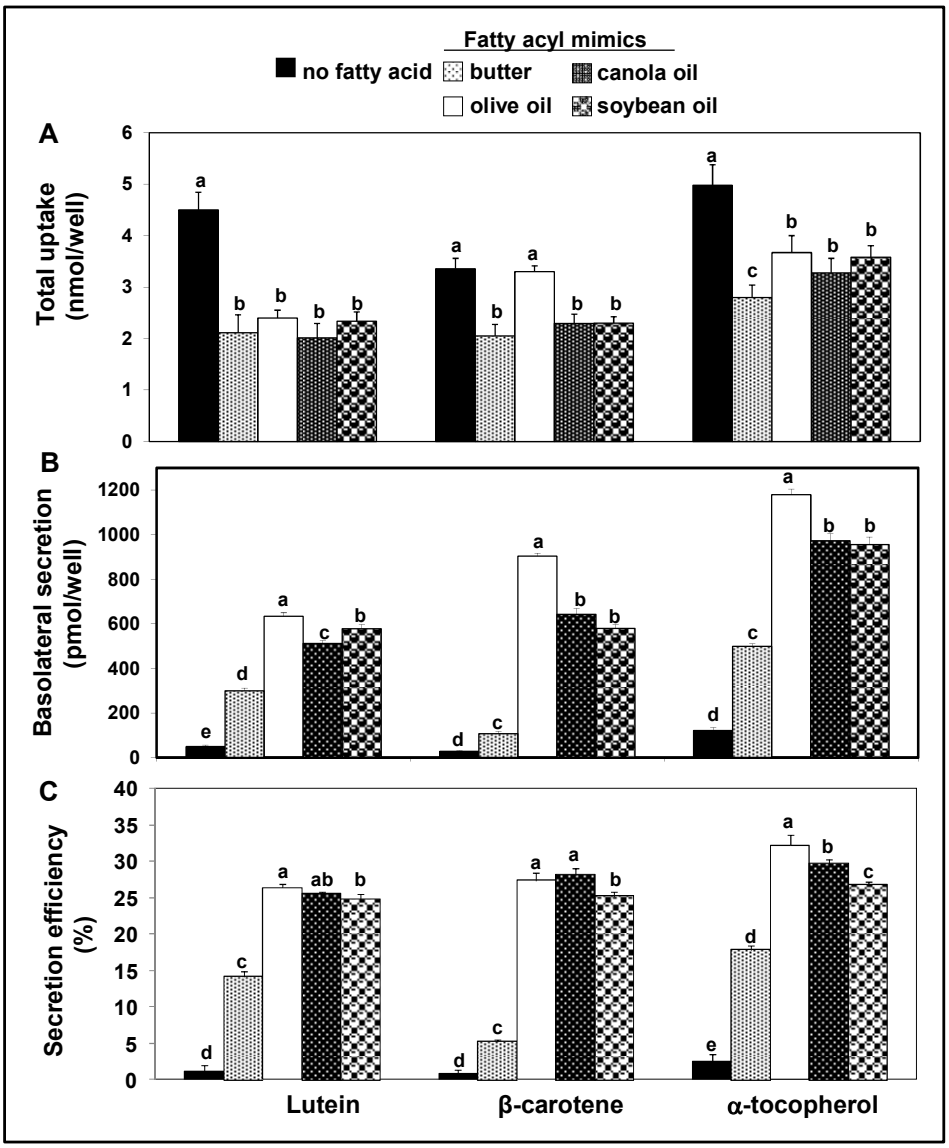
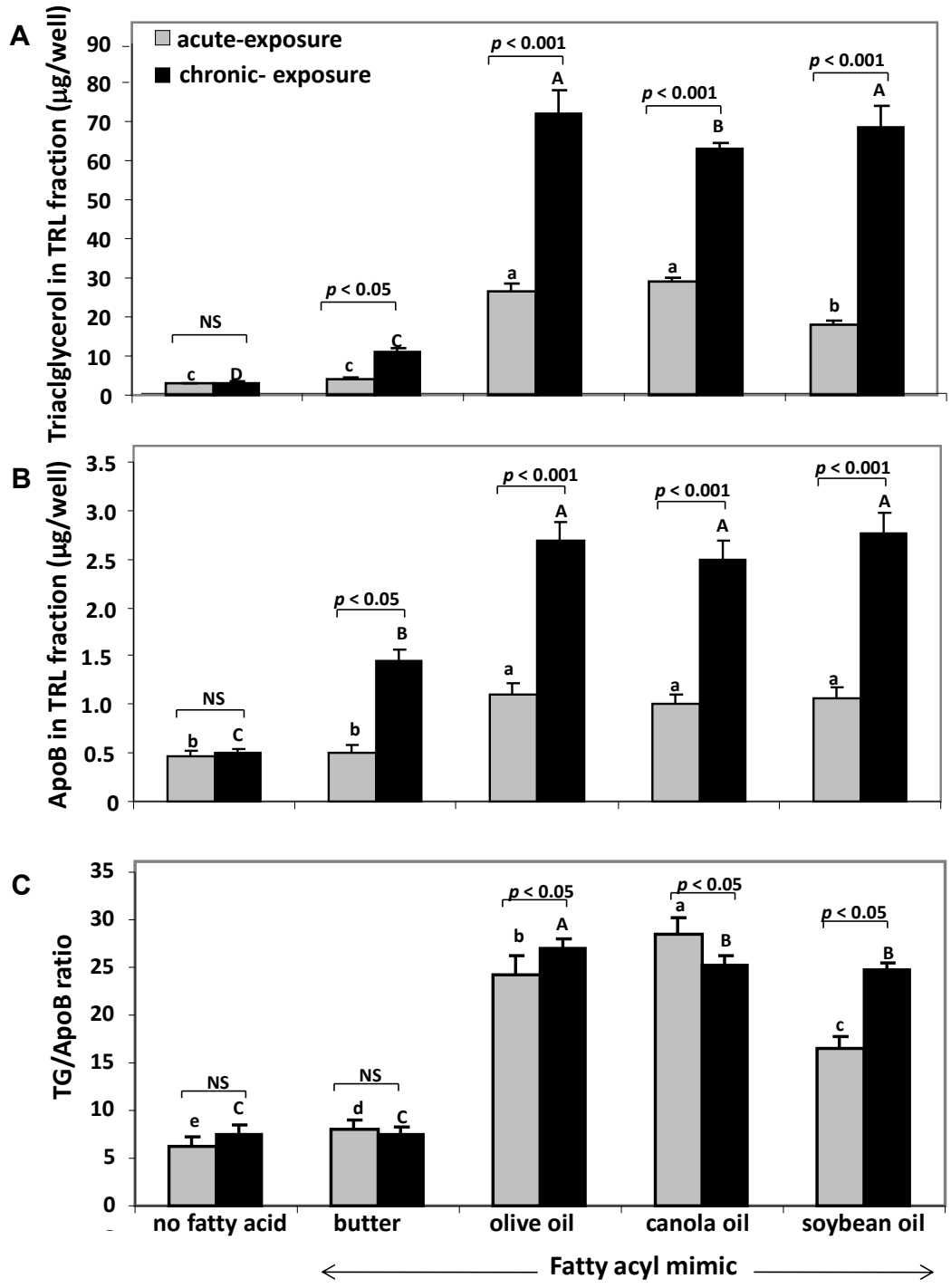

Fatty acyl mimics
no fatty acid
butter
canola oil
olive oil
soybean oil
A
Total uptake (nmol/well)
B
Basolateral secretion (pmol/well)
C
Secretion efficiency (%)
Lutein
β-carotene
α-tocopherol

Fig. 5


A
acute-exposure
chronic- exposure
Triacglycerol in TRL fraction (μg/well)
p < 0.001
p < 0.001
p < 0.001
p < 0.05
NS
B
ApoB in TRL fraction (μg/well)
p < 0.001
p < 0.001
p < 0.001
p < 0.05
NS
C
TG/ApoB ratio
p < 0.05
p < 0.05
p < 0.05
NS
NS
no fatty acid
butter
olive oil
canola oil
soybean oil
Fatty acyl mimic