Reprod Nutr Dev (1994) 34, 439-448

Original article

Effect of calcium and mineral waters on oleic-acid uptake by isolated hamster enterocytes

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(Received 15 February 1994; accepted 11 July 1994)

Summary — The effect of calcium upon the uptake of oleic acid solubilized with 10 mM taurocholate was investigated using an *in vitro* model of isolated enterocytes. The addition of Ca^{2+} to the incubation medium (Hanks' medium) led to a decrease in oleic-acid uptake. This uptake inhibition was dependent on both the amount of Ca^{2+} and the fatty-acid concentration, since the inhibitory effect was significant for 10 μM but not 100 μM oleic acid. The determination of the monomeric activity of oleic acid indicated that the decrease in fatty-acid uptake was not linked to the formation of insoluble calcium soaps. The replacement of Hank's medium by several mineral waters containing between 0.3 and 11.7 mM Ca^{2+} significantly reduced the uptake of both 10 and 100 μM oleic-acid. The ionic composition of these waters was correlated with the decrease of initial rate of oleic acid uptake, but Ca^{2+} and other ions could interfere by synergetic effects with the fatty-acid-absorption mechanism. It is concluded that the ion-induced inhibition of oleic-acid uptake is not due to the formation of insoluble soaps but rather to a direct effect on the fatty-acid transport in membranes. Whether the fatty-acid binding protein in the plasma membrane is involved in the effect of Ca^{2+} on fatty-acid transport remains to be established.

Ca^{2+} / mineral water / oleic acid / intestinal absorption / isolated enterocytes / hamster

Résumé — Influence du calcium et d'eaux minérales sur le captage de l'acide oléique par les entérocytes isolés du hamster. *L'influence du calcium sur le captage de l'acide oléique solubilisé par 10 mM de taurocholate est étudiée sur un modèle* in vitro *d'entérocytes isolés. L'addition de Ca^{2+} au milieu d'incubation (milieu de Hanks) induit une diminution du captage de l'acide oléique. Cette inhibition du captage dépend de la concentration en calcium ajouté, et de la concentration en acide gras puisque l'effet est significatif pour 10 μM mais pas pour 100 μM d'acide oléique. L'évaluation de l'activité monomérique de l'acide oléique suggère que l'inhibition du captage n'est pas liée à la formation de savons calciques insolubles. Le remplacement du milieu de Hanks par différentes eaux minérales apportant entre 0,3 et 11,7 mM de Ca^{2+} conduit à une diminution très significative du captage de l'acide oléique 10 et 100 μM. Les corrélations établies entre la composition ionique de ces eaux et la vitesse initiale de captage de l'acide gras indiquent que, outre Ca^{2+}, d'autres ions par un effet de synergie doivent inter-*

férer avec le mécanisme d'absorption de cet acide gras. En conclusion, l'inhibition du captage de l'acide oléique produite par les ions présents dans le milieu d'incubation n'est pas due à la formation de savons insolubles mais plutôt à un effet direct sur le transport entérocytaire, vraisemblablement lié à la protéine membranaire chargée du transport des acides gras à longue chaîne.

Ca^{2+} / eaux minérales / acide oléique / absorption intestinale / entérocytes isolés / hamster

INTRODUCTION

Several epidemiological studies indicate that the incidence of colon cancer is positively correlated with the dietary fat intake (Willett, 1989; Willett *et al,* 1990; Weisburger, 1991) and negatively correlated with the intake of calcium (Appleton *et al,* 1987; Sorenson *et al,* 1988). One mechanism by which calcium might reduce the effect of fat may be the formation of insoluble salts with bile acids and free fatty acids (Wargovich *et al,* 1983, 1984; Newmark *et al,* 1984; Lipkin and Newmark, 1985). It is generally admitted that soluble calcium (Ca^{2+}) in the intestinal lumen precipitates fatty acids leading to an increase in faecal fatty-acid excretion and a decrease in intestinal fatty acid absorption (Mattson *et al,* 1979; Appleton *et al,* 1992; Govers and Van der Meer, 1993). However, some studies have shown a significant stimulation of the rate of fatty-acid absorption by calcium ions (Strauss, 1977; Bernard *et al,* 1989; Saunders and Sillery, 1989). These conflicting results could be explained by the use of different experimental conditions which led to the proposal various mechanisms for intestinal fatty-acid uptake (simple diffusion, facilitated diffusion, and active transport). There are increasingly more arguments showing that the cell uptake of long-chain fatty acids occurs according to a carrier-mediated process. These results were obtained with *in vitro* studies using everted intestinal sacs or isolated perfused intestines (Chow and Hollander, 1978, 1979; Hollander *et al,* 1984; Molina *et al,* 1990). Studies using isolated intestinal cells indicate that oleic-acid uptake (Stremmel, 1988) and α-linolenic acid uptake (Goré and Hoinard, 1993) occur *via* a facilitated membrane translocation process, utilizing a fatty-acid binding protein in the membrane (Stremmel *et al,* 1985). The activity of this membrane transport protein could be modulated by extracellular medium, particularly its mineral composition.

We report here on the effect of calcium and other ions on the uptake of micellar solutions of oleic acid/taurocholate by isolated hamster intestinal cells. The effect of calcium on oleic-acid uptake was analysed: i) by adding $CaCl_2$ to the physiological incubation solution (Hanks' medium); or ii) by replacing the incubation medium by mineral waters containing different levels of ions, particularly calcium (0.3–11.7 mM).

MATERIALS AND METHODS

Animals

Fifteen adult male Syrian hamsters weighing 120–140 g (Elevage Dépré, Saint-Doulchard, France) were used. They were fed with a standard diet (ref 105, UAR, Villemoisson, France). Before experiments they were fasted overnight, but had free access to tap water.

Chemicals

Unless otherwise stated all chemicals were purchased from Sigma Chimie (Saint-Quentin-Fallavier, France). [1-^{14}C]Oleic acid (2.1 GBq/mmol) was purchased from New England Nuclear (Du Pont de Nemours, Paris, France).

The scintillation liquid Optiphase 'Hisafe' II was obtained from LKB (Pharmacia, Saint-Quentin-en-Yvelines, France). Solutions of labeled oleic acid were prepared by isotopic dilution.

Experimental procedure

Preparation of intestinal cells

Intestinal cells were isolated as previously described (Goré and Hoinard, 1987, 1989). Briefly, the hamsters were killed by diethyl ether overexposure (Scientist authorisation No 2897, ministère de l'Agriculture et de la Forêt, France) and the entire small intestine was removed, rinsed with oxygenated buffer solution (95% O_2, 5% CO_2) containing 27 mM sodium citrate, and incubated for 10 min in the same buffer at 37°C. It was then emptied, filled with oxygenated buffer solution containing 1.5 mM ethylenediaminetetraacetic acid, incubated for 3 min at 37°C and then gently finger palpated for 2 min. This treatment was repeated twice. The buffer containing intestinal cells was recovered in Hanks' solution. After centrifugation (765 *g*) the isolated cells were resuspended in Hanks' medium containing collagenase type IA (37°C, 15 min with stirring). The intestinal cells were then washed twice and resuspended in Hanks' medium (cellular stock solution containing about 5 mg protein/ml). Light microscopy of the suspension showed that about 90% of the cells displayed the typical features of villus-tip cells. Viability was assessed by trypan blue exclusion capacity and lactate dehydrogenase (EC 1.1.1.27) release assay, using lactate dehydrogenase optimized kit No DG1340-K, Sigma diagnostics (Sigma Chimie, Saint-Quentin-Fallavier, France). Protein concentration was assayed with the method of Lowry *et al* (1951).

Preparation of solutions

Mineral water

Four commercial mineral waters (Volvic, Evian, Vittel and Contrex) containing between 0.3 and 11.7 mM Ca^{2+} and commonly used in French diets, were chosen for this study. Table I shows the mineral content of these waters and indicates a very low osmolarity. Because intestinal cells were resuspended in these waters in the experimental protocol, it was necessary to adjust the medium osmolarity and pH at physiological values (*ie* 290 mOsm/kg H_2O and pH 7.3). This was done by adding mannitol (240–265 mM) and Hepes (10 mM) to these waters.

Working cellular suspensions

Six millilitres of cellular stock solution were centrifuged (765 *g*, 2 min) and the pellet was resuspended in 6 ml of control medium (Hanks' medium) or 6 ml of mineral water (working cellular suspension containing about 5 mg protein/ml).

Table I. Composition* of mineral waters and Hanks' medium.

Ions (mg/l)	*Water 1 (Volvic)*	*Water 2 (Evian)*	*Water 3 (Vittel)*	*Water 4 (Contrex)*	*Hanks' medium*
Ca^{2+}	9.9 (0.26 mM)	78 (1.95 mM)	202 (5.05 mM)	467 (11.67 mM)	50 (1.26 mM)
Mg^{2+}	6.1	24	36	84	19.7
Na^{+}	9.4	5.0	3.0	7.0	3 164
K^{+}	5.7	1.0	–	3.0	227
Cl^{-}	8.4	4.5	–	7.0	5 139
SO_4^{2-}	6.9	10	306	1 192	79
HCO_3^{-}	65.3	357	402	377	0
Total ion	109	309	750	1 850	9 790
pH	7.0	7.2	7.4	7.2	7.3
Osmolarity (mOms/kg H_2O)	1	6	11	24	276

* Compositions determined by the manufacturer.

Fatty-acid solutions

Five millilitres of fatty-acid solution were prepared by isotopic dilution of [1-^{14}C]oleic acid (20 μM or 200 μM) in Hanks' medium, Hanks' medium plus $CaCl_2$ (6 mM or 20 mM), or mineral water; each medium contained 20 mM sodium taurocholate. Solubilization was obtained by sonication for 5 min in a Ney 300 water-bath sonicator (Bioblock Scientific, Illkirch, France).

Determination of oleic-acid uptake

Uptake of [1-^{14}C]oleic acid was measured by rapid vacuum filtration assay (Stremmel, 1988). One millilitre of the working cellular suspension was incubated for 1 min in a 37°C water bath with stirring and uptake was started by the addition of 1 ml of fatty-acid solution (medium plus sodium taurocholate) containing [1-^{14}C]oleic acid at 37°C. At various time intervals over the initial 20-s incubation period, 200 μl sample aliquots (about 0.5 mg protein) were pipetted into 3 ml of 5 mg albumin/ml in Hanks' medium (4°C) to stop cellular influx and to remove bound fatty acids. The stop solution with cells was pipetted onto the center of Whatman GF/C glass microfibre filter (Poly Labo, Strasbourg, France), and filtered under 8 x 10^4 Pa vacuum pressure using a filtration apparatus (Hoefer model FH225V, Bioblock, Illkirch, France). Cells were washed with 5 ml of 5 mg albumin/ml solution (4°C) and thereafter with 20 ml of Hanks' medium (4°C). Filters were placed in scintillation vials, and 4 ml of scintillation liquid was added. Radioactivity was determined in a LKB-1215 Rackbeta liquid scintillation counter (Pharmacia, Saint-Quentin-en-Yvelines, France). Nonspecific radioactivity binding to filters and cells was measured in each experiment by adding 5 mg albumin/ml in Hanks' medium (4°C) before the addition of corresponding aliquots of cells and [1-^{14}C]oleic-acid working solutions. This background was < 5% of the total radioactivity measured. The value of each sample was the net radioactivity after subtraction of this blank.

Determination of oleic-acid monomer activity

The technique used to determine monomer activity is based upon the partitioning of fatty acids between aqueous solution and a solid organic phase, a polyethylene disc. With this system, Sallee (1974) demonstrated that monomer activities of solutions of long-chain fatty acids dissolved in bile acid were linearly dependent on the total fatty-acid concentration in the incubation medium. Polyethylene discs, 1.2 cm in diameter, were punched from 0.16 mm thick polyethylene film manufactured by Roth Sochiel (Lauterbourg, France). To remove any oil and debris, the discs were washed in methanol and distilled water and were dried before used. After equilibration of 2 discs in 3 ml fatty-acid test solutions for 24 h (shaking water bath, 37°C) the discs were removed, rinsed in Hanks' medium and the radioactivity associated with the discs was determined.

Statistical analysis

Results are expressed as means ± SE. Initial rates of uptake (V_i) were calculated by the method of least squares, which allows an estimation of simple linear regression parameters and their standard deviations. Comparisons of ordinates at origin with zero value were evaluated by Student's *t*-test. Curves characterizing the intracellular uptake of oleic acid were calculated with Microsoft Excel solver (Les Ulis, France). Comparisons between group means were evaluated by the unpaired *t*-test. Statistical significance of differences among more than 2 groups were determined by analysis of variance (PCSM programs, Deltasoft, Grenoble, France).

RESULTS

The aim of this work was to study the influence of calcium on the intestinal absorption of oleic acid. The dispersion of fatty acids in the incubation medium is one of the indispensable conditions for studying their uptake by intestinal cells. Some authors have used bovine serum albumin to complex fatty acids (Stremmel, 1988; Potter *et al,* 1989; Trotter and Storch, 1991). During the intestinal absorption process, fatty acids were physiologically solubilized by bile salts present in the lumen. Thus, we chose to use sodium taurocholate (final concentration 10 mM) as a dispersing agent in this study.

Cell viability

Because bile salts have been shown to affect the viability or permeability of intestinal cells (Westergaard and Dietschy, 1976; Buset *et al*, 1990), we tested the effect of 10 mM taurocholate on the cells. In the Hanks' medium without taurocholate, initial cell viability was about 85%. The addition of 10 mM taurocholate led to a decrease of cellular viability of 1.5 and 2% per min when assessed by trypan blue exclusion and lactate dehydrogenase release, respectively. Because incubation time with taurocholate was < 1 min (see *Materials and methods*) cellular viability loss resulting from the presence of taurocholate was not significant. In addition, prior work in our laboratory has shown that permeability was not significantly modified by taurocholate (Goré and Hoinard, 1993).

Kinetics of intracellular oleic-acid uptake

Figure 1 shows an example of absorption kinetics for 100 μM oleic acid solubilized in 10 mM taurocholate. During the first 20 s of incubation the rate of uptake was maximal and linear. The initial rate of uptake (V_i) was determined by linear regression fit from uptake measured over the initial 20-s incubation period. Statistical tests indicated no significant difference between ordinate at origin and the zero value.

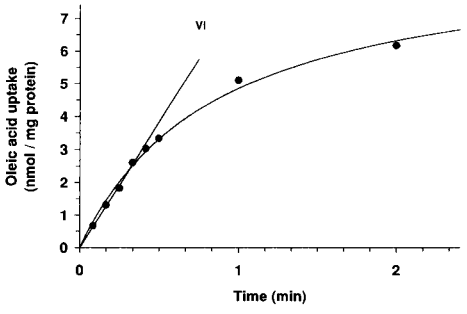

Fig 1. An example of the time course of oleic-acid uptake by isolated enterocytes at 37°C. [1-^{14}C] Oleic acid solubilized in taurocholate (final concentrations 100 μM and 10 mM, respectively) was added to cellular suspension (final protein concentration 2.5 mg/ml). Initial rate of uptake (V_i) was calculated by linear regression fit from uptake measured over the initial 20-s incubation period.

Effect of the incubation medium on cellular uptake of oleic acid

Table II shows that when the oleic-acid monomer was solubilized in 10 mM sodium taurocholate, its activity was not significantly modified by the addition of calcium to the incubation medium. Similarly, replacement of incubation medium by the mineral waters with higher ionic concentrations (particularly in calcium) did not lead to any changes in monomeric activity. Table III, however, indicates that the addition of $CaCl_2$ to the control incubation medium (Hanks' medium) induced a significant decrease in 10 μM oleic-acid cellular uptake. The effect of $CaCl_2$ was dependent on the amount of Ca^{2+} added to the medium (inhibition of 19 and 32% for 3 and 10 mM $CaCl_2$, respectively). Although a reduction in fatty-acid uptake was noted, the intestinal absorption of 100 μM oleic acid was not significantly modified by $CaCl_2$. On the other hand, replacement of Hanks' medium by mineral waters drastically decreased the cellular uptake of both 10 and 100 μM oleic acid (mean inhibition 60 ± 16%). It can be noted that the decrease of oleic-acid uptake induced by mineral waters was even greater than those obtained with 10 mM $CaCl_2$. Statistical analysis revealed that the inhibition of fatty-acid uptake was different from one water to the others. The examination of the mineral content of these waters (see table I) leads to 2 remarks: i) in comparison to the Hanks' medium, all the waters used in this study were Na^+-, K^+- and Cl^--depleted; and ii) the amounts of Ca^{2+}, Mg^{2+}, SO_4^{2-} and HCO_3^- increased from water 1 to water 4 (*ie* Volvic < Evian < Vittel < Contrex). Table IV

Table II. Effect of Ca^{2+} and water 4 (Contrex) on oleic-acid content of polyethylene discs.

Medium	n	*Polyethylene disc content (pmol/disc)*	
		Oleic acid (10 μM)	*Oleic acid (100 μM)*
Hanks' (control)	8	448 ± 9	6 174 ± 153
Hanks' + $CaCl_2$ 3 mM	7	423 ± 11	5 748 ± 152
Hanks' + $CaCl_2$ 10 mM	7	542 ± 36	4 616 ± 355
Water 4 (Contrex)	8	549 ± 15	5 288 ± 670

Values are means ± SE of *n* experiments. Incubation medium contained 10 or 100 μM oleic acid solubilized in 10 mM taurocholate. There is no statistical difference ($P > 0.05$) between the means for oleic acid 10 μM, and for oleic acid 100 μM (analysis of variance).

Table III. Effect of Ca^{2+} and mineral water on oleic-acid uptake (10 and 100 μM) by isolated hamster enterocytes.

Medium	n	*Initial rate of uptake (pmol/mg protein/min)*	
		Oleic acid (10 μM)	*Oleic acid (100 μM)*
Hanks' (control)	40	1 853 ± 59	7 385 ± 401
Hanks' + $CaCl_2$ 3 mM	8	1 506 ± 53[a]	6 927 ± 229
Hanks' + $CaCl_2$ 10 mM	8	1 260 ± 48[ab]	6 469 ± 306
Water 1 (Volvic)	8	978 ± 35[a]	4 815 ± 132[a]
Water 2 (Evian)	8	700 ± 23[a]	3 840 ± 162[a]
Water 3 (Vittel)	8	591 ± 16[a]	2 607 ± 99 [a]
Water 4 (Contrex)	8	230 ± 8[a]	2 474 ± 142[ac]

Values are means ± SE of n experiments. Incubation medium contained 10 or 100 μM oleic acid solubilized in 10 mM taurocholate. Comparisons to control (Hanks' medium) [a] $P < 0.001$. Comparison between $CaCl_2$ 3 mM and 10 mM, [b] $P < 0.01$. Comparison between water 3 and water 4, [c] $P > 0.05$, no statistical difference.

indicates that total ion content of the waters was statistically inversely correlated to the uptake of oleic acid ($r = -0.948$, $P < 0.001$ and $r = -0.811$, $P < 0.001$ for oleic acid uptake 10 and 100 μM, respectively). The ions Ca^{2+}, Mg^{2+} and SO_4^{2-} especially seem to play an inhibitory role in oleic acid cellular uptake ($-0.969 < r < -0.824$; $P < 0.001$). In order to clarify this point, some complementary experiments were conducted (table V). First, the influence of Cl^- or SO_4^{2-} was apparently negligible since no important change in oleic-acid uptake was demonstrated when $CaSO_4$ was substituted for $CaCl_2$, or when NaCl (140 mM) was added to Na^+-depleted water (water 4). This addition of Na^+ led to a significant increase of oleic-acid uptake, which was insufficient for control level recovery. Second, the addition of 10 mM EGTA, which

Table IV. Correlation table between ionic composition of mineral waters and initial rate of uptake (V_i) of 10 and 100 μM oleic acid.

	Total ion	*Ca^{2+}*	*Mg^{2+}*	*SO_4^{2-}*	*HCO_3^-*	*K^+*	*Na^+*	*Cl^-*
Total ion	**1**							
Ca^{2+}	**0.998**	**1**						
Mg^{2+}	**0.993**	**0.994**	**1**					
SO_4^{2-}	**0.991**	**0.982**	**0.975**	**1**				
HCO_3^-	0.570	0.613	0.636	0.458	**1**			
K^+	−0.19	−0.222	−0.302	−0.088	**−0.88**	**1**		
Na^+	−0.1	−0.156	−0.15	0.03	**−0.84**	**0.999**	**1**	
Cl^-	0.056	0.023	−0.06	0.158	−0.737	**0.970**	**0.977**	**1**
V_i (10 μM)	**−0.948**	**−0.958**	**−0.969**	**−0.906**	−0.757	0.439	0.323	0.210
V_i (100 μM)	**−0.811**	**−0.842**	**−0.824**	−0.736	**−0.866**	0.483	0.631	0.258

The correlation coefficients in bold type are highly significant ($P < 0.001$).

Table V. Effect of the incubation medium on 10 μM oleic-acid uptake by isolated hamster enterocytes.

Medium	n	*Initial rate of uptake (pmol/mg protein/min)*
Hanks' (control)	24	1 693 ± 37
Hanks' + $CaCl_2$ 10 mM	8	1 466 ± 56[a]
Hanks' + $CaSO_4$ 10 mM	8	1 532 ± 39[ac]
Water 4 (Contrex)	16	625 ± 23[b]
Water 4 (Contrex) + 10 mM EGTA	8	763 ± 28[bd]
Water 4 (Contrex) + 140 mM NaCl	8	823 ± 16[bd]

Values are means ± SE of *n* experiments. Incubation medium contained 10 μM oleic acid solubilized in 10 mM taurocholate. Comparisons to control (Hanks' medium), [a] $P < 0.01$, [b] $P < 0.001$. Comparisons between $CaCl_2$ and $CaSO_4$, [c] $P > 0.05$, no statistical difference. Comparison between water 4 and water 4 + EGTA, or water 4 + NaCl, [d] $P < 0.001$.

induced a decrease of Ca^{2+} content of water 4 (Contrex) from 11.7 to 1.3 mM, could not restore the oleic-acid uptake measured in the control.

DISCUSSION

The results obtained in the first part of this study show that when calcium was added to intestinal cell incubation medium, the uptake of oleic acid (18:1*n*–9) was reduced. The extent of this effect increased as the calcium concentration in the medium was raised. For the same calcium concentration, the decrease of uptake was greater at low concentration of fatty acids (table III). A Ca^{2+}/fatty acid molar ratio > 100 is required before decreased oleic-acid uptake was noted. Similar results were obtained with

linolenic acid (18:3*n*–3), a long-chain polyunsaturated fatty acid (data not shown). This agrees with the reduction in long-chain fatty-acid uptake brought about by calcium as reported by several authors (Mattson *et al,* 1979; Appleton *et al,* 1992; Govers *et al,* 1993). In the present study, the decrease in oleic-acid uptake induced by calcium was not directly related to the formation of insoluble calcium soaps. As indicated by the determination of monomeric activities, the fatty-acid monomer, which is the only form easily absorbed by intestinal cells (Westergaard and Dietschy, 1976), was not modified by the addition of calcium (table II). This lack of formation of insoluble products could depend on our experimental conditions (oleic acid dissolved in 10 mM taurocholate, pH 7.30). Jandacek (1991) has shown that the solubility of calcium oleate is 40–50 mg/l (about 1 200 μM), *ie* much higher than the concentrations used in the present work. In addition, calcium has relatively little effect on the absorption of unsaturated fatty acids (Mattson *et al,* 1979; Guéguen, 1992) and oleic acid itself would cause the solubilization of calcium-oleate salts (Jandacek, 1991). Finally, the presence of taurocholate could modify the Ca^{2+}-fatty-acid interaction (Van der Meer *et al,* 1990; Gu *et al,* 1992; Govers and Van der Meer, 1993). Calcium thus appears to interfere directly or indirectly with the fatty-acid-uptake mechanisms of intestinal cells, rather than through a reduction in the concentration of free fatty acid that can be absorbed. Whether Ca^{2+} reduces oleate uptake through modifications in the membrane fluidity (Jacobson and Papahadjopoulos, 1975; Baciulis *et al,* 1992) and/or in the activity of the membrane fatty-acid binding protein which transports long-chain fatty acids into intestinal cells (Stremmel, 1988; Goré and Hoinard, 1993) cannot be assessed from the present study.

The results of the second part of this study, involving the effect of mineral water on oleic-acid uptake, are not so straightforward to interpret. Overall, replacing the incubation medium used as control (Hanks' medium) by mineral water led to an approximately 60% reduction of fatty-acid uptake. This inhibition of transport was noted with both 10 and 100 μM oleic acid. Intestinal cell uptake of long-chain fatty acids requires the presence of Na^+ in the incubation medium (Stremmel, 1988; Goré and Hoinard, 1993) and the mineral waters used in this study were highly depleted in Na^+ (table I). This could in part explain their effect. Nevertheless, the addition of 140 mM NaCl to these waters did not reestablish the rate of oleate uptake as in control conditions. The decrease in initial rates of oleic-acid uptake (V_i) was correlated with the overall inorganic composition of the water used (table IV). The relationship between the reduction in V_i and the ionic composition of the mineral water was highly significant for Mg^{2+}, Ca^{2+} and SO_4^{2-} (probably because of $CaSO_4$ and $MgSO_4$ present in the water). On its own, the calcium concentration of these waters (between 0.3 and 11.7 mM) does not explain the considerable decrease observed in oleate uptake. Even though complexing Ca^{2+} with EGTA caused a significant increase in oleic-acid uptake (table V), the control values cannot be reached. It thus seems that the action of mineral water on the uptake of oleic acid by intestinal cells can be ascribed to the simultaneous and synergetic presence of several ions (notably Ca^{2+}, Mg^{2+}) (Behling *et al,* 1990) and absence of others (notably Na^+, Cl^-).

A study involving the effect of these different ions (in particular Ca^{2+} and Mg^{2+}) on fatty-acid-transport-protein coupling is currently under way by our group and should furnish more precise responses on the mechanism(s) of inhibition reported here.

ACKNOWLEDGMENT

This study was supported by a grant from BSN group (France).

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