Effect of different long-chain fatty acids on cholecystokinin release in vitro and energy intake in free-living healthy males

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Abstract

Long-chain fatty acids have been shown to suppress appetite and reduce energy intake (EI) by stimulating the release of gastrointestinal hormones such as cholecystokinin (CCK). The effect of NEFA acyl chain length on these parameters is not comprehensively understood. An in vitro screen tested the capacity of individual NEFA (C12 to C22) to trigger CCK release. There was a gradient in CCK release with increasing chain length. DHA (C22) stimulated significantly (P < 0·01) more CCK release than all other NEFA tested. Subsequently, we conducted a randomised, controlled, crossover intervention study using healthy males (n 18). The effects of no treatment (NT) and oral doses of emulsified DHA-rich (DHA) and oleic acid (OA)-rich oils were compared using 24 h EI as the primary endpoint. Participants reported significantly (P = 0·039) lower total daily EI (29 % reduction) with DHA compared to NT. There were no differences between DHA compared to OA and OA compared to NT. There was no between-treatment difference in the time to, or EI of, the first post-intervention eating occasion. It is concluded that NEFA stimulate CCK release in a chain length-dependent manner up to C22. These effects may be extended to the in vivo setting, as a DHA-based emulsion significantly reduced short-term EI.

Key words: Secretin tumour cells-1: Long-chain fatty acids: Cholecystokinin: Energy intake

Food ingestion stimulates satiety and inhibits energy intake (EI) via complex mechanisms, in which signals arising from the gastrointestinal tract play a critical role(31). The presence of dietary fat in the gastrointestinal tract, for example, has been shown to trigger the endogenous release of many anorectic hormones including cholecystokinin (CCK)(1–3). CCK is secreted from I-cells in the duodenum(3). Dietary fats are hydrolysed from TAG into monoacylglycerols and NEFA by lingual, gastric and pancreatic lipase before absorption. Luminal NEFA (rather than TAG) trigger CCK release and longer-chain, unsaturated NEFA appear to be the most potent stimulants(4–7). Matzinger et al.(9) suggest that the generation of long-chain NEFA via lipid hydrolysis is a critical step in fat-induced inhibition of EI and that this signal is mediated via CCK release and CCK-A receptors(9). Basal human plasma CCK concentrations are generally in the low picomolar (approximately 1 pm) range but can increase 10-fold following high-fat meals(9). It is hypothesised that CCK regulates appetite by inhibiting gastric emptying and promoting gastric mechanoreceptor stimulation(9).

The secretin tumour cell (STC)-1 enteroendocrine cell line provides a model to study intestinal hormone expression since it demonstrates some features of native intestinal hormone-secreting cells. It has been used to show acyl chain length-dependent NEFA-induced CCK release up to C18(5,10). In the present investigation, we tested the effects of NEFA, with longer chain lengths than previously reported, on CCK release. The NEFA most effective at stimulating CCK in vitro was incorporated into an oil emulsion to test its effects on in vivo 24 h EI.

Experimental methods

Cholecystokinin release cell assay

Reagents were from Sigma (Poole, UK) unless otherwise stated. STC-1 cells were kindly supplied by Dr Rumio,
University of Milan. Cells were maintained as previously described\(^{11}\) and seeded into twelve-well plates, at 6 × 10\(^3\) per well, 48 h before incubation with solutions of docosaneoic (12 : 0), cis-9-octadecenoic (18 : 1), all cis-9,12-octadecadienoic (18 : 2), cis-9, trans-11-octadecadienoic (18 : 2), cis-5,9,12-octadecatrienoic (18 : 3), all cis-5,8,11,14,17-eicosapentaenoic (20 : 5) and all cis-4,7,10,13,16,19-docosahexaenoic (22 : 6) at final concentrations of 66 nM, for 60 min. Supernatant levels of CCK were assayed by ELISA according to the manufacturer's protocol (Phoenix Europe GmbH, Karlsruhe, Germany). CCK concentrations were normalised against total cellular protein by quantifying the cell lysates according to the manufacturer's protocol (Thermo Scientific, Rockford, IL, USA).

**Prospective food intake in response to consumption of long-chain fatty acids-based emulsions**

**Subjects.** A total of eighteen subjects were recruited via email and poster advertisements around the University of Sheffield. Exclusion criteria included: BMI outside normal and overweight UK categories (<20 and >29·9 kg/m\(^2\)), recent food poisoning, diagnosed gastrointestinal, metabolic or endocrine disorders, food allergies, use of nutritional supplements, smoking, age <18 and >65 years and having implantable electronic devices (contraindicated for bioelectrical impedance analysis). Height and weight were recorded to the nearest 0·1 cm and 0·1 kg, respectively, to calculate BMI to the nearest 0·1 kg/m\(^2\). Bioelectrical impedance analysis was undertaken using BodyStat 1500 (BodyStat Limited, Isle of Man, British Isles). Body fat and lean weight were recorded to the nearest 0·1 % or 0·1 kg, respectively.

**Study design.** This was a controlled, single-blind, three-way crossover study designed to examine the effects of two long-chain fatty acid emulsions, compared to no treatment (NT), on prospective food intake. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Sheffield (SMBRER158). Written informed consent was obtained from all subjects. Treatments were orally administered, low-energy (145 kJ (35 kcal)), 45 % protein, carbohydrate and fat contents of these meals. There was an informed consent that NEFA-induced CCK secretion in STC-1 cells is directly proportional to chain length, but only NEFA up to C18 have been examined\(^{5,10,15}\). Our \textit{in vitro} results extend the literature by demonstrating that this chain length relationship exists from C12 up to at least C22. Our data also show that CCK secretion, triggered by C18 NEFA, differed by degree of unsaturation. These preliminary findings may indicate that the NEFA-mediated CCK-response may be sensitive to both desaturation and double-bond configuration.

**Results**

**Effects of NEFA on \textit{in vitro} cholecystokinin release**

The NEFA elicited significantly (\(P < 0·01\)) different degrees of CCK release both using absolute quantification and when normalised for biomass. The order of NEFA-induced CCK production was DHA (22 : 6), EPA 20 : 5), linoleic acid (18 : 3), linolenic acid (18 : 2), oleic acid (18 : 1), conjugated linoleic acid (18 : 2) and lauric acid (12 : 0). The ethanol control assayed 1·16 ng/g (Fig. 1). Post \textit{hoc} comparisons indicated that DHA was significantly (\(P < 0·01\)) more effective at stimulating CCK release in STC-1 cells than any other NEFA tested.

**Effect of DHA, oleic acid and no treatment on prospective food intake and time to first eating occasion**

All glycaemic measurements were within the normal fasting range\(^{14}\). There was no difference between treatments in time to first post-intervention eating occasion or the energy, protein, carbohydrate and fat contents of these meals. There was a significant (\(P = 0·048\)) difference in total daily EI across treatments. Post \textit{hoc} comparisons indicated that subjects reported significantly (\(P = 0·039\)) lower EI with DHA relative to NT. There was no difference in total daily EI for DHA compared to OA or OA compared to NT (Table 1). Although reduction in the intake of all macronutrients accounted for the fall in EI, treatment did not affect the intake of any individual macronutrient.

**Discussion**

This study shows that DHA increases the \textit{in vitro} release of CCK and decreases EI in healthy men. Several studies show that NEFA-induced CCK secretion in STC-1 cells is directly proportional to chain length, but only NEFA up to C18 have been examined\(^{5,10,15}\). Our \textit{in vitro} results extend the literature by demonstrating that this chain length relationship exists from C12 up to at least C22. Our data also show that CCK secretion, triggered by C18 NEFA, differed by degree of unsaturation. These preliminary findings may indicate that the NEFA-mediated CCK-response may be sensitive to both desaturation and double-bond configuration.
DHA was the most effective NEFA at stimulating CCK release and this led to an assessment of its effects on prospective food intake in fifteen healthy men. In this study, DHA significantly reduced EI by 3558 kJ compared to NT. If this level of EI reduction was sustained in the longer term, it could lead to weight loss. A study testing such effects in overweight subjects is warranted. OA yielded a non-significant reduction in EI compared to NT. The EI reduction must be attributable to a specific physiological response as DHA and OA were isoenergetic. Other researchers suggest that the effect of long-chain NEFA on appetite is partly mediated by increased CCK production\(^{16,17}\). STC-1 cells and EE cells in vivo are, however, stimulated to release a cascade of appetite-suppressing hormones in response to lipids\(^{17–19}\). It cannot therefore be assumed that the EI reduction observed here was mediated solely by CCK.

DHA did not affect the first post-treatment eating occasion; EI was suppressed throughout the day. This suggests that the time course by which fatty acids affect food consumption could extend up to 24 h. Food ingestion stimulates the release of numerous hormones into the blood, some of which have a direct effect on gastric emptying and others have longer-lasting postprandial effects on satiety\(^{18}\). Other studies have reported greater fullness and reduced hunger after consuming high compared to low DHA/EPA diets relative to olive oil\(^{20}\) and reduced EI up to 48 h after consumption of high-polyunsaturated compared to high-MUFA meals\(^{21}\).

Comparing the effects of equienergetic oils eliminates the possibility that EI alteration is mediated by energy and/or total lipid ingestion, and suggests that the effects are due to differences in the oils’ fatty acid composition. OA was demonstrated to have some bioactivity in the in vitro experiment and this was reflected in vivo. Other studies using oil containing predominantly OA have also observed effects suggesting that it is not biologically inert\(^{22,23}\). Evidence suggests that emulsification of fish oils improves digestion and absorption

![Fig. 1. Cholecystokinin (CCK) release from secretin tumour cells (STC)-1 in response to NEFA; ng CCK release from STC-1 cells per µg protein. Values are means and standard deviations of three experiments (±2% of the mean). All NEFA produced significantly (P < 0.01) more CCK than the ethanol control except lauric acid. Post hoc comparisons using the Tukey honestly significant difference test indicate that all NEFA stimulated significantly (P < 0.01) different quantities of CCK production compared to each other. CLA, conjugated linoleic acid.](image)

Table 1. Fasting glycaemia, time to first eating occasion, energy, protein, carbohydrate and fat intakes for the first post-intervention eating occasion (≤ 210 kJ) and total study day (post-intervention to 23.59 hours) by treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NT (Mean)</th>
<th>SD</th>
<th>OA (Mean)</th>
<th>SD</th>
<th>DHA (Mean)</th>
<th>SD</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glycaemia (mmol/L)</td>
<td>5.4</td>
<td>0.36</td>
<td>5.3</td>
<td>0.59</td>
<td>5.5</td>
<td>0.47</td>
<td>F: 0.474, P: 0.626</td>
</tr>
<tr>
<td>Time to first eating occasion (min)</td>
<td>96.0</td>
<td>64.0</td>
<td>133.0</td>
<td>99.0</td>
<td>121.0</td>
<td>64.0</td>
<td>F: 0.908, P: 0.411</td>
</tr>
<tr>
<td>First eating occasion</td>
<td>3148</td>
<td>1984</td>
<td>3035</td>
<td>1733</td>
<td>2507</td>
<td>1302</td>
<td>F: 0.616, P: 0.545</td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>12,097</td>
<td>5429</td>
<td>9912</td>
<td>2733</td>
<td>8539</td>
<td>2720</td>
<td>F: 3.262, P: 0.048†</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>111</td>
<td>36.0</td>
<td>102</td>
<td>39.0</td>
<td>87</td>
<td>37.0</td>
<td>F: 1.564, P: 0.221</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>314</td>
<td>129</td>
<td>273</td>
<td>79.0</td>
<td>259</td>
<td>87.0</td>
<td>F: 1.219, P: 0.306</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>110</td>
<td>48.0</td>
<td>102</td>
<td>37.0</td>
<td>77</td>
<td>27.0</td>
<td>F: 2.935, P: 0.064</td>
</tr>
</tbody>
</table>

NT, no treatment; OA, oleic acid.

† Mean value was significantly different from that of DHA (P = 0.039, post hoc comparisons using the Tukey honestly significant difference test).

† There was significant difference in total daily EI across treatments (P = 0.039).
of n-3 long-chain polyunsaturated NEFA due to solubility modification\(^1\).\(^2\). Using our novel oil-emulsion approach may offer greater study standardisation and limit between study variance caused by differential meal response.

Overall, our results show that NEFA can increase appetite hormone CCK release in vitro and this effect is chain-length-dependent. Compared to NT, 24 h EI was reduced after consumption of a predominantly DHA (22:5) oil-emulsion, but not after a predominantly OA (18:0) oil-emulsion. Further studies to examine if reduced EI is sustained and leads to weight loss are warranted, as are evaluations in female and obese subjects.

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