

**Stimulation of CCK and GLP-1 secretion and expression in STC-1 cells by
human jejunal contents and *in vitro* gastrointestinal digests from casein
and whey proteins**

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1. SDS-PAGE ANALYSIS

The methodology is described in the manuscript

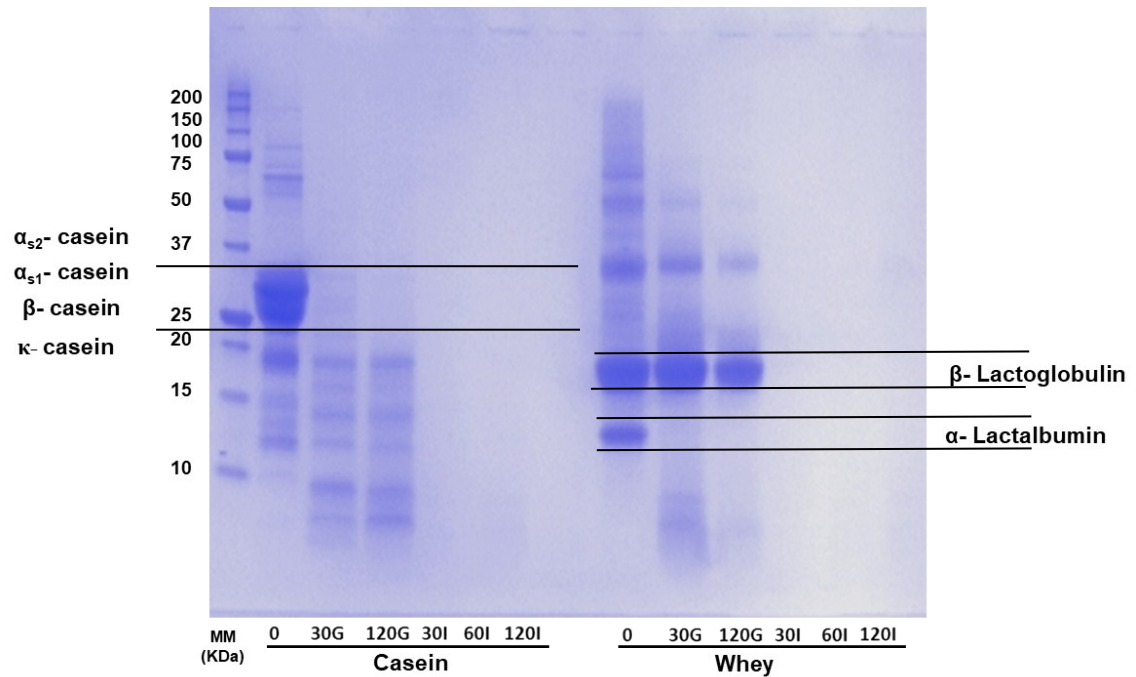


Figure S1: SDS-PAGE analysis of casein and whey *in vitro* gastrointestinal digests. Each lane corresponds to a different time points of gastric (G) and gastrointestinal (I) digestion. MM, molecular weight marker; 30G, 30 min gastric digestion; 120G, 120 min gastric digestion; 30I, 30 min gastrointestinal digestion; and 120I, 120 min gastrointestinal digestion.

2. Analysis by HPLC-tandem mass spectrometry

Table S1. β -casein-derived peptides identified in casein *in vitro* digests taken at 30 and 120 min of the gastric phase, and 30 and 120 min of the intestinal phase

Range	Sequence	Range	Sequence
1 5	RELEE	48 58	KIHPFAQTQSL
1 6	RELEEL	55 86	TQSLVYFPFGPIPNLSLQNIPLTQTTPVVVPP
1 11	RELEELNVPGE	57 62	SLVYPF
2 8	ELEELNV	57 66	SLVYFPFGPI
5 17	ELNVPGEIVESLS	57 68	SLVYFPFGPIPN
5 34	ELNVPGEIVESLSSEESITRINKKIEKFQ	58 66	LVYFPFGPI
6 12	LNVPGEI	58 68	LVYFPFGPIPN
6 14	LNVPGEIVE	59 66	VYFPFGPI
6 16	LNVPGEIVESL	59 68	VYFPFGPIPN
6 26	LNVPGEIVESLSSEESITRI	59 80	VYFPFGPIPNLSLQNIPLTQT
7 13	NVPGEIV	60 66	YFPFGPI
7 14	NVPGEIVE	60 68	YFPFGPIPN
7 15	NVPGEIVES	61 68	PFPFGPIPN
7 16	NVPGEIVESL	61 71	PFPFGPIPNSLP
7 35	NVPGEIVESLSSEESITRINKKIEKFQS	61 78	PFPFGPIPNLSLQNIPLTQT
7 41	NVPGEIVESLSSEESITRINKKIEKFQSEEQQT	67 74	PNSLQNI
8 37	VPGEIVESLSSEESITRINKKIEKFQSEE	69 80	SLPQNIPLTQT
9 14	PGEIVE	69 82	SLPQNIPLTQTPV
9 26	PGEIVESLSSEESITRI	70 80	LPQNIPLTQT
11 20	EIVESLSSE	71 93	PQNIPLTQTTPVVVPPFLQPEVM
12 24	IVESLSSEESIT	73 80	NIPPLTQT
16 39	LSSSEESITRINKKIEKFQSEEQQ	73 82	NIPPLTQTPV
17 26	SSSEESITRI	73 92	NIPPLTQTPVVVPPFLQPEV
32 38	KFQSEEQ	75 82	PPLTQTPV
33 38	FQSEEQ	76 83	PLTQTPVV
33 39	FQSEEQQ	78 82	TQTPV
33 40	FQSEEQQQ	78 85	TQTPVVVP
34 39	QSEEQQ	78 87	TQTPVVVPPF
35 44	SEEQQTEDE	81 92	PVVVPPFLQPEV
36 61	KVNELSKDIGSESTEDQAMEDIKQME	81 93	PVVVPPFLQPEVM
40 45	QTEDEL	83 91	VVPPFLQPE
40 55	LSKDIGSESTEDQAME	83 92	VVPPFLQPEV
41 45	TEDEL	85 92	PPFLQPEV
41 47	TEDELQD	91 99	EVMGVSKVK
41 48	TEDELQDK	92 99	VMGVSKVK
42 47	EDELQD	92 100	VMGVSKVKE
43 48	DELQDK	100 105	EAMAPK
45 58	LQDKIHPFAQTQSL	108 113	EMPPFK
46 55	QDKIHPFAQT	108 116	EMPPFKYPV
46 58	QDKIHPFAQTQSL	114 118	YPVEP
47 55	DKIHPFAQT	114 119	YPVEPF
47 74	ESTEDQAMEDIKQMEAESISSSEIIVPN	117 136	EPFTESQSLTLTDVENLHLP

Range	Sequence	Range	Sequence
118 126	PFTESQSLT	159 167	PQSVLSLSQ
119 125	FTESQSL	159 184	PQSVLSLSQSKVLPVPQKAVPYPQRD
125 140	LTLTDVENLHLPPLL	160 168	QSVLSLSQS
125 142	LTLTDVENLHLPPLLQS	161 171	SVLSLSQSKVL
126 132	TLTDVEN	162 170	VLSLSQSKV
126 140	TLTDVENLHLPPLL	162 172	VLSLSQSKVLP
126 142	TLTDVENLHLPPLLQS	162 190	GAWYVPLGTQYTDAPSFSDIPNPIGSEN
127 132	LTDVEN	164 173	SLSQSKVLPV
127 139	LTDVENLHLPPL	164 188	SLSQSKVLPVPQKAVPYPQRDMPIQ
127 140	LTDVENLHLPPLL	164 189	SLSQSKVLPVPQKAVPYPQRDMPIQA
127 142	LTDVENLHLPPLLQS	164 190	SLSQSKVLPVPQKAVPYPQRDMPIQAF
128 140	TDVENLHLPPLL	164 191	SLSQSKVLPVPQKAVPYPQRDMPIQAFLL
128 142	TDVENLHLPPLLQS	164 192	SLSQSKVLPVPQKAVPYPQRDMPIQAFLL
128 146	TDVENLHLPPLLQSWMHQ	165 172	LSQSKVLP
129 138	DVENLHLP	168 196	SKVLPVPQKAVPYPQRDMPIQAFLLYQEP
129 139	DVENLHLPPL	170 175	VLPVPQ
130 138	VENLHLP	173 188	VPQKAVPYPQRDMPIQ
134 139	HLPLPL	177 182	AVPYPQ
134 141	HLPLPLLQ	184 189	DMPIQA
141 163	QSWMHQPHQPLPPTVMFPPQSVL	189 205	AFLLYQEPVLGPPVRGPF
141 170	QSWMHQPHQPLPPTVMFPPQSVLSLSQSKV	190 209	FLLYQEPVLGPPVRGPFPIIV
143 156	WMHQPPLPPTVM	191 209	LLYQEPVLGPPVRGPFPIIV
144 154	MHQPPLPPT	192 201	LYQEPVLGPV
145 154	HQPPLPPT	192 209	LYQEPVLGPPVRGPFPIIV
147 154	PHQPPLPPT	193 197	YQEPV
149 154	QPLPPT	193 198	YQEPVL
154 164	TVMFPPQSVLS	193 201	YQEPVLGPV
156 162	MFPQSV	193 208	YQEPVLGPPVRGPFPII
157 162	FPPQSV	193 209	YQEPVLGPPVRGPFPIIV
157 163	FPPQSVL	194 201	QEPVLGPV
157 165	FPPQSVLSL	196 209	PVLGPPVRGPFPIIV
		202 207	RGPFPI

Table S2. α_{s1} - casein-derived peptides identified in casein *in vitro* digests taken at 30 and 120 min of the gastric phase, and 30 and 120 min of the intestinal phase

Range	Sequence	Range	Sequence
1 16	RPKHPIKHQGLPQEV	70 74	EIVPN
1 20	RPKHPIKHQGLPQEVLNENL	83 88	KEDVPS
1 23	RPKHPIKHQGLPQEVLNENLLRF	84 89	EDVPSE
2 11	PKHPIKHQGL	87 95	PSERYLGYL
6 12	IKHQGLP	87 104	PSERYLGYLEQLLRLLKKY
8 13	HQGLPQ	96 114	EQLLRLLKKYKVPQLEIVPN
12 19	PQEVLNEN	97 103	QLLRLLKK
16 20	LNENL	98 129	LLRLLKKYKVPQLEIVPNSAEERLHSMKEGIHA
17 23	NENLLRF	99 109	LRLKKYKVPQL
24 30	FVAPFPE	100 129	RLKKYKVPQLEIVPNSAEERLHSMKEGIHA
24 31	FVAPFPEV	102 107	KKYKVP
24 33	FVAPFPEVFG	103 107	KYKVP
24 39	FVAPFPEVFGKEKVNE	104 109	YKVPQL
25 30	VAPFPE	105 112	KVPQLEIV
25 31	VAPFPEV	109 114	LEIVPN
25 33	VAPFPEVFG	109 118	LEIVPNSAEE
25 39	VAPFPEVFGKEKVNE	110 118	EIVPNSAEE
26 39	APFPEVFGKEKVNE	111 118	IVPNSAEE
27 31	PFPEV	112 118	VPNSAEE
27 39	PFPEVFGKEKVNE	114 119	NSAEER
29 39	PEVFGKEKVNE	120 125	LHSMKE
33 39	GKEKVNE	124 131	KEGIHAQQ
35 39	EKVNE	125 129	EGIHA
35 45	EKVNELSKDIG	128 134	HAQQKEP
36 41	KVNELS	133 138	EPMIGV
38 44	NELSKDI	133 140	EPMIGVNQ
40 47	LSKDIGSE	135 145	MIGVNQELAYF
40 56	LSKDIGSESTEDQAMED	136 141	IGVNQE
41 77	SKDIGSESTEDQAMEDIKQMEAESISSSEEIVPNSVE	136 149	IGVNQELAYFYPEL
43 51	DIGSESTED	138 145	VNQELAYF
43 52	DIGSESTEDQ	142 149	LAYFYPEL
43 53	DIGSESTEDQA	143 149	AYFYPEL
45 51	GSESTED	144 149	YFYPEL
45 69	GSESTEDQAMEDIKQMEAESISSSE	154 161	YQLDAYPS
53 58	AMEDIK	154 164	YQLDAYPSGAW
54 79	MEDIKQMEAESISSSEEIVPNSVEQK	155 161	QLDAYPS
55 63	EDIKQMEAE	157 164	DAYPSGAW
58 66	KQMEAESIS	160 168	PSGAWYYVP
59 64	QMEAES	160 181	PSGAWYYVPLGTQYTDAPSFSD
60 66	MEAESIS	160 190	PSGAWYYVPLGTQYTDAPSFSDIPNPIGSEN
64 75	SISSSEEIVPNS	161 170	SGAWYYVPLG
65 92	ISSSEEIVPNSVEQKHQKEDVPSERYL	165 172	YYVPLGTQ
66 73	SSSEEIVP	165 179	YYVPLGTQYTDAPSF
69 77	EEIVPNSVE	166 196	YVPLGTQYTDAPSFSDIPNPIGSENSEKTTM

Range		Sequence	Range		Sequence
168	178	PLGTQYTDAPS	177	183	PSFSDIP
171	191	TQYTDAPSFSDIPNPIGSENS	178	186	SFSDIPNPI
172	178	QYTDAPS	179	187	FSDIPNPIG
173	178	YTDAPS	180	184	SDIPN
173	179	YTDAPSF	180	186	SDIPNPI
173	181	YTDAPSFSD	180	196	SDIPNPIGSENSEKTTM
173	191	YTDAPSFSDIPNPIGSENS	180	199	SDIPNPIGSENSEKTTMPLW
174	180	TDAPSF	189	195	ENSEKTT
174	186	TDAPSFSDIPNPI	190	198	NSEKTTMPL

Table S3. β -lactoglobulin-derived peptides identified in whey protein *in vitro* digests taken at 120 min of the gastric phase, and 30 and 120 min of the intestinal phase.

Range	Sequence	Range	Sequence
1 8	LIVTQTMK	42 49	YVEELKPT
1 10	LIVTQTMKGL	42 51	YVEELKPTPE
1 14	LIVTQTMKGLDIQK	42 54	YVEELKPTPEGDL
2 6	IVTQT	42 55	YVEELKPTPEGDLE
2 8	IVTQTMK	42 57	YVEELKPTPEGDLEIL
2 9	IVTQTMKG	42 59	YVEELKPTPEGDLEILLQ
2 10	IVTQTMKGL	42 60	YVEELKPTPEGDLEILLQK
2 11	IVTQTMKGLD	43 49	VEELKPT
3 8	VTQTMK	43 51	VEELKPTPE
4 11	TQTMKGLD	43 52	VEELKPTPEG
6 12	TMKGLDI	43 54	VEELKPTPEGDL
9 20	GLDIQKVAGTWY	43 55	VEELKPTPEGDLE
9 23	GLDIQKVAGTWYSLA	43 57	VEELKPTPEGDLEIL
10 18	LDIQKVAGT	44 51	EELKPTPE
10 19	LDIQKVAGTW	44 54	EELKPTPEGDL
11 19	DIQKVAGTW	44 60	EELKPTPEGDLEILLQK
12 19	IQKVAGTW	45 51	ELKPTPE
12 28	IQKVAGTWYSLAMAASD	45 54	ELKPTPEGDL
15 20	VAGTWY	45 55	ELKPTPEGDLE
15 26	VAGTWYSLAMAA	46 51	LKPTPE
21 26	SLAMAA	46 54	LKPTPEGDL
22 31	LAMAASDISL	47 51	KPTPE
23 32	AMAASDISLL	47 55	KPTPEGDLE
24 41	MAASDISLLDAQSAPLRV	47 56	KPTPEGDLEI
26 32	ASDISLL	48 56	PTPEGDLEI
26 41	ASDISLLDAQSAPLRV	50 55	PEGDLE
27 32	SDISLL	55 62	EILLQKWE
27 39	SDISLLDAQSAPL	56 67	ILLQKWENDECA
27 41	SDISLLDAQSAPLRV	57 70	LLQKWENDECAQKK
27 42	SDISLLDAQSAPLRVY	58 69	LQKWENDECAQK
28 54	DISLLDAQSAPLRVYVEELKPTPEGDL	58 74	LQKWENDECAQKKIIAE
30 39	SLLDAQSAPL	60 66	KWENDEC
30 41	SLLDAQSAPLRV	60 67	KWENDECA
32 38	LDAQSAP	61 66	WENDEC
32 41	LDAQSAPLRV	61 67	WENDECA
33 39	DAQSAPL	61 68	WENDECAQ
33 40	DAQSAPLR	61 73	WENDECAQKKIIA
33 41	DAQSAPLRV	62 67	ENDECA
33 42	DAQSAPLRVY	63 69	NDECAQK
34 38	AQSAP	68 76	QKKIIAEKT
40 57	RVYVEELKPTPEGDLEIL	69 77	KKIIAEKTK
41 48	VYVEELKP	73 91	AEKTKIPAVFKIDALNENK
41 57	VYVEELKPTPEGDLEIL	74 80	EKTKIPA

Range		Sequence	Range		Sequence
74	82	EKTKIPAVF	124	131	RTPEVDDE
75	82	KTKIPAVF	124	138	RTPEVDDEALEKFDK
76	82	TKIPAVF	125	130	TPEVDD
77	82	KIPAVF	125	131	TPEVDDE
77	90	KIPAVFKIDALNEN	125	132	TPEVDDEA
82	91	FKIDALNENK	125	134	TPEVDDEALE
82	92	FKIDALNENKV	125	135	TPEVDDEALEK
83	89	KIDALNE	125	136	TPEVDDEALEKF
83	91	KIDALNENK	125	137	TPEVDDEALEKFD
83	92	KIDALNENKV	125	138	TPEVDDEALEKFDK
83	93	KIDALNENKVL	125	139	TPEVDDEALEKFDKA
83	102	KIDALNENKVLVLDTDYKKY	125	140	TPEVDDEALEKFDKAL
85	91	DALNENK	125	141	TPEVDDEALEKFDKALK
86	95	ALNENKVLVL	126	131	PEVDDE
89	97	ENKVLVLDT	126	132	PEVDDEA
90	95	NKVLVL	126	133	PEVDDEAL
92	100	VLVLDTDYK	127	134	EVDDEALE
92	101	VLVLDTDYKK	127	135	EVDDEALEK
96	102	DTDYKKY	127	136	EVDDEALEKF
96	103	DTDYKKYL	128	133	VDDEAL
96	104	DTDYKKYLL	128	138	VDDEALEKFDK
105	116	FCMENSAEPEQS	129	141	DDEALEKFDKALK
106	114	CMENSAEPE	137	143	DKALKAL
107	112	MENSAE	142	148	ALPMHIR
107	114	MENSAEPE	148	156	RLSFNPTQL
107	116	MENSAEPEQS	148	160	RLSFNPTQLEEQC
107	117	MENSAEPEQSL	148	162	RLSFNPTQLEEQCHI
107	118	MENSAEPEQSLV	149	154	LSFNPT
107	119	MENSAEPEQSLVC	149	156	LSFNPTQL
108	113	ENSAEP	149	159	LSFNPTQLEEQ
108	114	ENSAEPE	149	160	LSFNPTQLEEQC
108	116	ENSAEPEQS	149	162	LSFNPTQLEEQCHI
109	117	NSAEPEQSL	150	154	SFNPT
110	116	SAEPEQS	150	156	SFNPTQL
110	118	SAEPEQSLV	150	159	SFNPTQLEEQ
112	117	EPEQSL	150	160	SFNPTQLEEQC
112	121	EPEQSLVCQC	150	162	SFNPTQLEEQCHI
122	141	LVRTPEVDDEALEKFDKALK	152	162	NPTQLEEQCHI
123	130	VRTPEVDD	153	162	PTQLEEQCHI
123	131	VRTPEVDDE	154	161	TQLEEQCH
123	132	VRTPEVDDEA	155	159	QLEEQ
123	133	VRTPEVDDEAL	155	160	QLEEQC
123	141	VRTPEVDDEALEKFDKALK	156	161	LEEQCH
124	130	RTPEVDD	157	161	EEQCH
			157	162	EEQCHI

Table S4. α -lactalbumin-derived peptides identified in whey proteins *in vitro* digests taken at 120 min of the gastric phase, and 30 and 120 min of the intestinal phase.

Range		Sequence	Range		Sequence
1	9	EQLTKCEVF	59	64	IWCKDD
1	11	EQLTKCEVFRE	59	73	IWCKDDQNPSSNIC
4	9	TKCEVF	61	68	CKDDQNP
8	14	VFRELKD	62	77	KDDQNPSSNICNISC
10	17	RELKDLKG	62	79	KDDQNPSSNICNISCDK
10	18	RELKDLKGY	63	68	DDQNP
12	16	LKDLK	69	76	SSNICNIS
13	20	KDLKGYGG	69	78	SSNICNISCD
15	35	LKGYGGVSLPEWVCTTFHTSG	74	80	NISCDKF
19	25	GGVSLPE	74	88	NISCDKFLDDDLTDD
19	27	GGVSLPEWV	75	88	ISCDKFLDDDLTDD
19	28	GGVSLPEWVC	76	83	SCDKFLDD
19	29	GGVSLPEWVCT	76	85	SCDKFLDDDL
19	30	GGVSLPEWVCTT	80	97	FLDDDLTDDIMCVKKILD
19	31	GGVSLPEWVCTTF	81	87	LDDDLTD
19	35	GGVSLPEWVCTTFHTSG	81	88	LDDDLTDD
19	36	GGVSLPEWVCTTFHTSGY	82	88	DDDLTDD
19	40	GGVSLPEWVCTTFHTSGYDTQA	82	89	DDDLTDDI
21	30	VSLPEWVCTT	83	89	DDLTDI
22	29	SLPEWVCT	89	97	IMCVKKILD
24	28	PEWVC	89	103	IMCVKKILDKVGINY
24	29	PEWVCT	90	103	MCVKKILDKVGINY
24	30	PEWVCTT	91	103	CVKKILDKVGINY
24	31	PEWVCTTF	92	99	VKKILDKV
24	35	PEWVCTTFHTSG	92	121	VKKILDKVGINYWLAHKALCSEKLDQWLCE
24	39	PEWVCTTFHTSGYDTQ	94	99	KILDKV
24	40	PEWVCTTFHTSGYDTQA	94	103	KILDKVGINY
26	31	WVCTTF	95	103	ILDKVGINY
26	35	WVCTTFHTSG	97	102	DKVGIN
30	40	TFHTSGYDTQA	97	103	DKVGINY
32	39	HTSGYDTQ	97	104	DKVGINYW
32	40	HTSGYDTQA	98	103	KVGINY
40	49	AIVQNNDSTE	104	117	WLAHKALCSEKLDQ
40	52	AIVQNNDSTEYGL	105	117	LAHKALCSEKLDQ
41	48	IVQNNDST	106	117	AHKALCSEKLDQ
41	49	IVQNNDSTE	106	119	AHKALCSEKLDQWL
41	50	IVQNNDSTEY	109	117	ALCSEKLDQ
41	51	IVQNNDSTEYG	110	117	LCSEKLDQ
41	52	IVQNNDSTEYGL	111	117	CSEKLDQ
45	51	NDSTEYG	111	118	CSEKLDQW
53	57	FQINN	117	123	QWLCEKL
53	58	FQINNK	118	123	WLCEKL

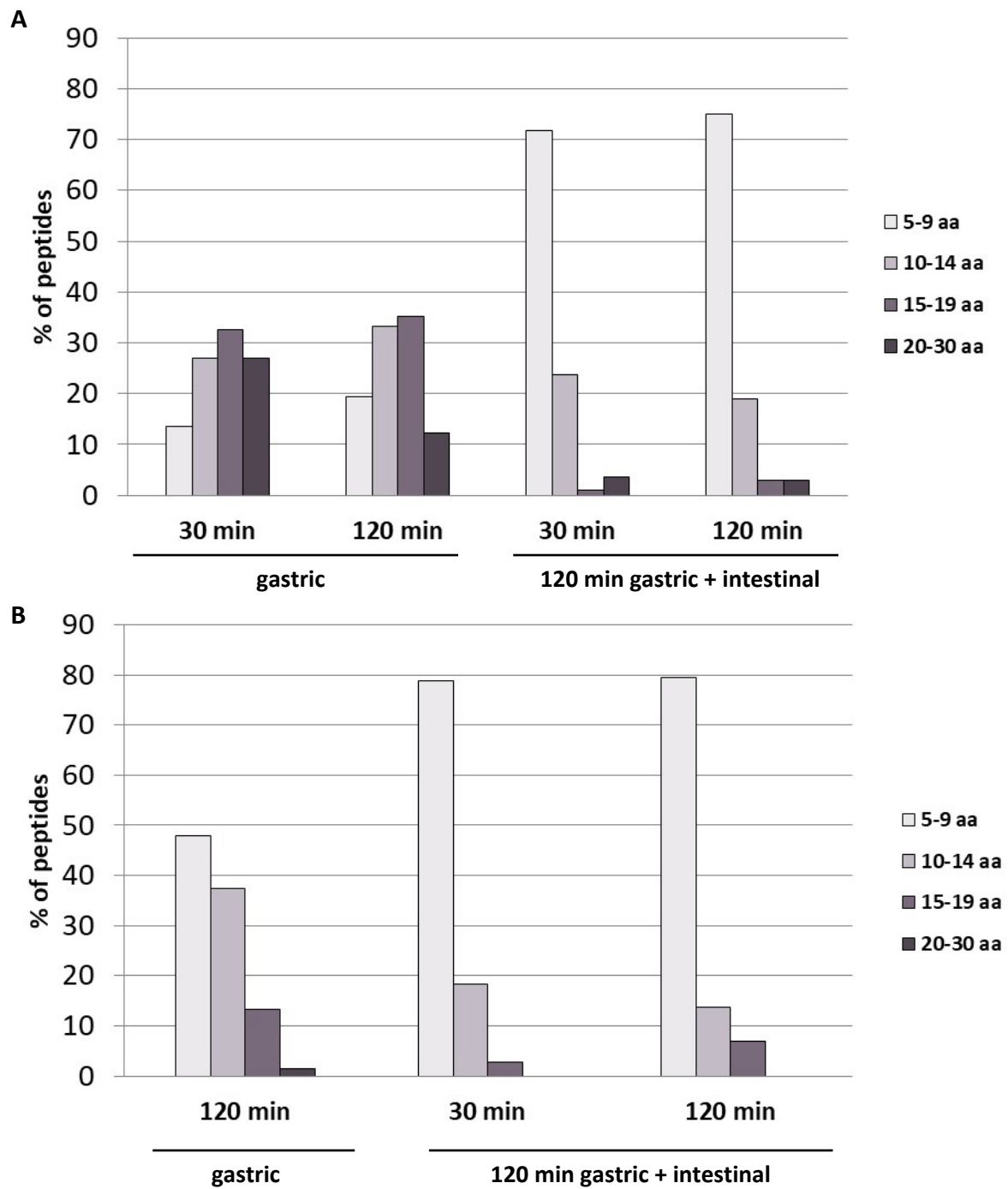


Figure S2: Size of peptides identified by HPLC-tandem mass spectrometry in (A) casein and (B) whey protein *in vitro* digests. The peptide size is expressed as number of amino acids (aa) and represented as percentage of the total identified peptides (y-axis) at each digestion time. Gastric digestion at 30 min is not shown due to the low hydrolysis degree in this sample. Each colour corresponds to a different peptide size range.

3. Comparison of CCK and GLP-1 secretion between *in vitro* and *in vivo* digests

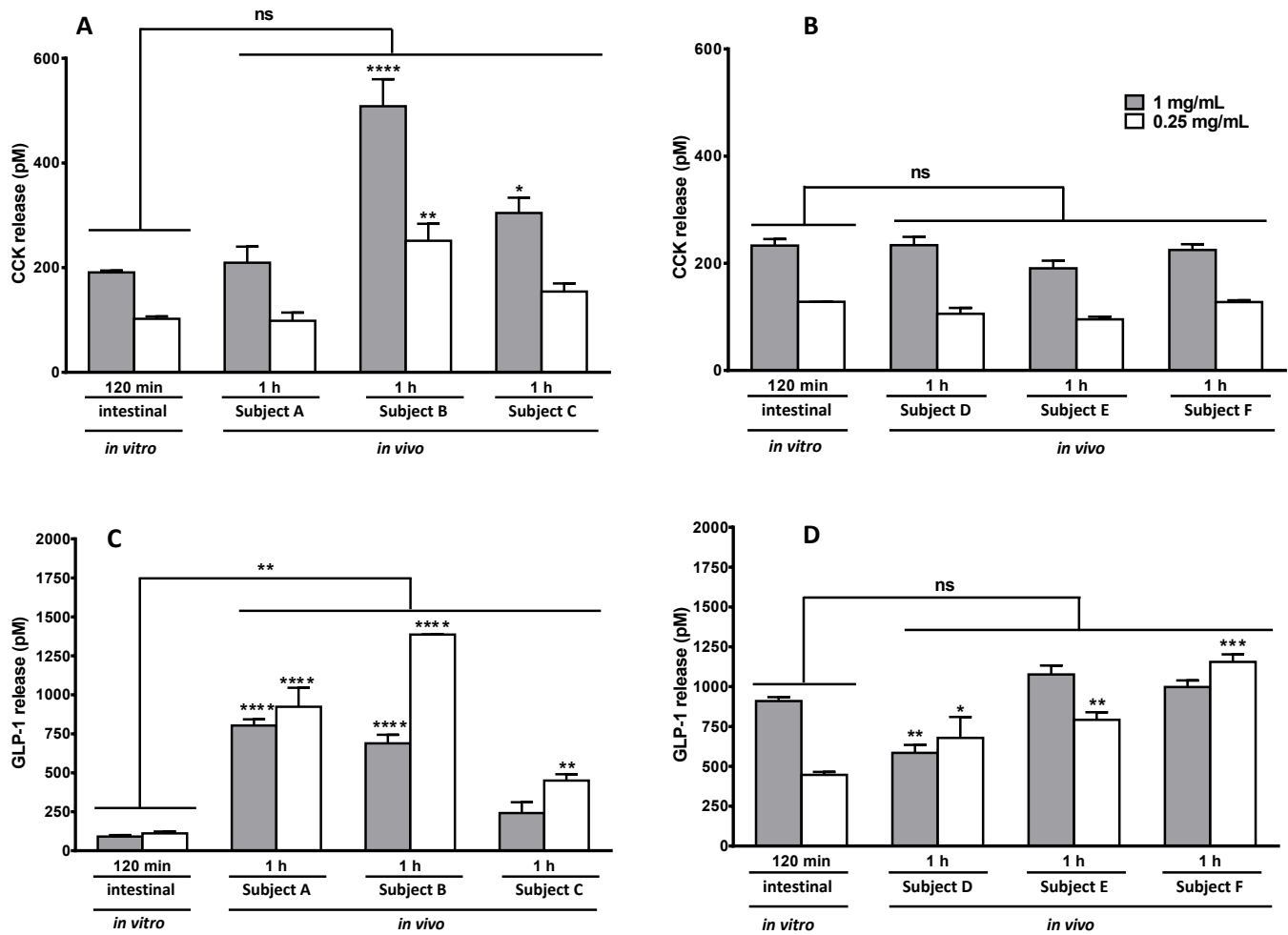


Figure S3: CCK secretion after 2 h incubation of STC-1 cells with (A) casein and (B) whey protein *in vitro* digests at the end of the digestion protocol (intestinal phase 120 min) and human jejunal effluents (*in vivo* digests) taken at 1 h after oral administration of the same substrates. Samples were incubated at two different protein concentrations, 1 and 0.25 mg/mL. GLP-1 secretion after 2 h incubation of STC-1 cells with (C) casein and (D) whey protein *in vitro* and *in vivo* digests. CCK and GLP-1 secretion was determined by ELISA. Error bars indicate SEM (n=3). Comparisons between samples tested at the same protein concentration and group comparisons were performed by two way ANOVA with Bonferroni post hoc test and is indicated by *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.