



MilkQua

Milk Quality along the Dairy
Chain for a Safe and
Sustainable MILK

WP5 – System biology assessment of the effects of essential oils after in vitro and in vivo studies

Fabrizio Ceciliani



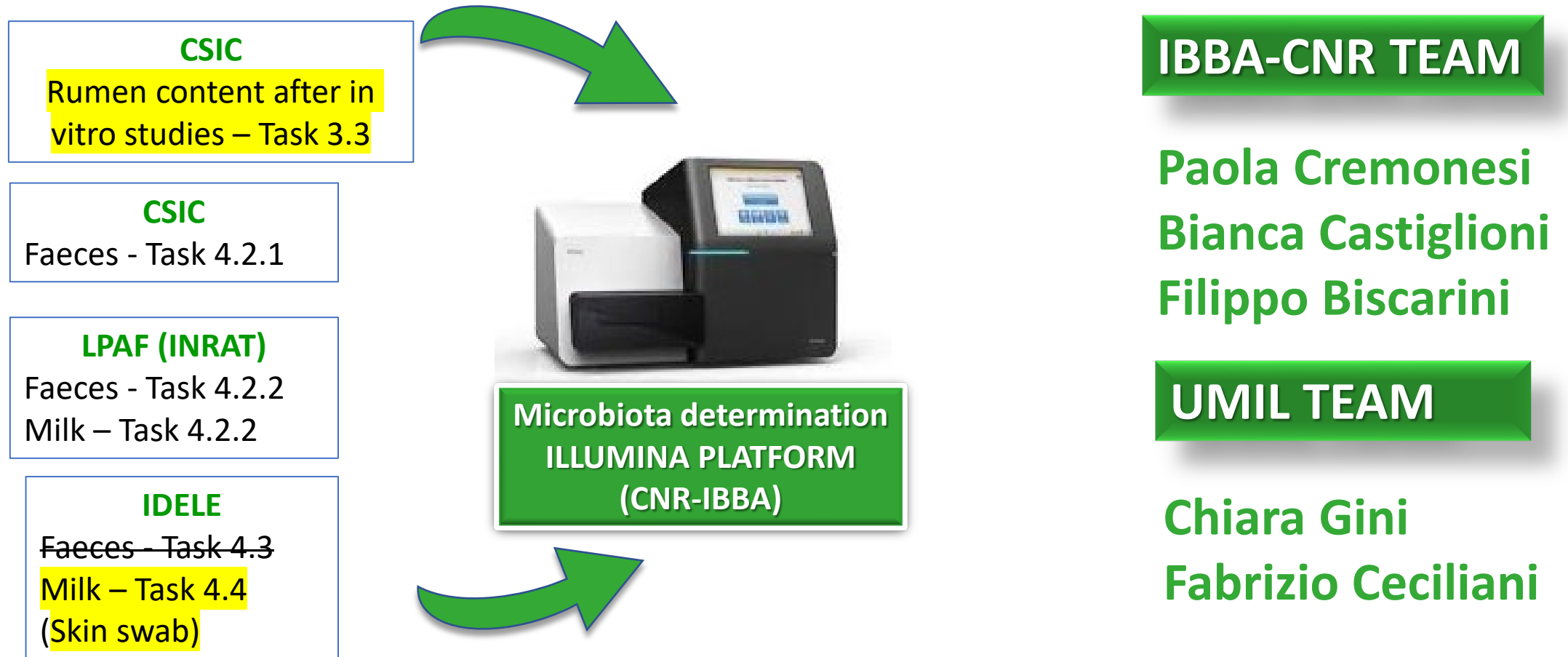
The PRIMA programme is supported under Horizon 2020, the European Union's Framework Programme for Research and Innovation



What we planned to deliver to the project

- 💧 D5.1 A list of bacterial at genus level that are present in faeces and milk of cows fed with different EOs, and from in vitro samples from WP4
- 💧 D5.2 The miRNome of milk as related to feeding with different EOs
- 💧 D5.3 A list of milk metabolites involved in the immune response which are modified after different EOs regimen.
- 💧 D5.4 A list of differentially abundant proteins after feeding with different EOs

D5.1 A list of bacterial at genus level that are present in faeces and milk of cows fed with different EOs, and from in vitro samples from WP4



D5.1 A list of bacterial at genus level that are present in faeces and milk of cows fed with different EOs, and from in vitro samples from WP4

- 💧 Milk, ruminal and swab samples sequenced (sequence available) – CNR platform IBBA-LODI
- 💧 Faecal samples: first delivery from Spain lost.
- 💧 Bioinformatic ongoing (CNR- platform/ Bioinformatic platform)
- 💧 First results?

~~December/January 2021/2022~~

From November 2021

D5.2 The miRNome of milk as related to feeding with different EO

LPAF (INRAT)
Milk – Task 4.2.2

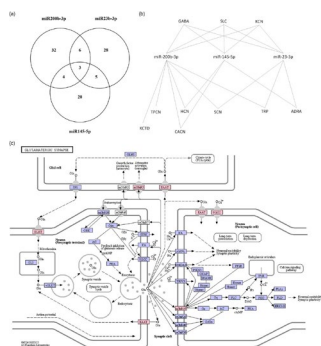
IDELE
Milk – Task 4.4



**microRNAome determination
ILLUMINA PLATFORM**

Gene ID	logFC	logCPM	Pvalue	FDR
20_10028	8.52238	9.80387	1.14E-11	8.21E-09
1_444	3.213022	7.85935	2.28E-11	8.03E-09
10_1014	3.873615	6.19521	8.59E-10	2.02E-07
6_10719	7.84926	3.96765	3.37E-08	5.44E-06
25_21482	2.38772	10.2107	4.71E-08	6.63E-06
12_1774	4.8588	8.26511	1.72E-07	2.02E-05
12_1004	4.78023	4.13532	2.42E-07	2.43E-05
26_22331	4.60624	3.85133	3.98E-07	3.48E-05
4_17023	-2.38303	5.14379	1.74E-06	0.000108
25_24537	2.320406	5.64271	2.02E-06	0.000142
22_10388	7.027623	2.63696	2.88E-06	0.000179
17_11082	4.014311	4.00666	9.63E-06	0.000228
18_12375	5.97322	3.36033	9.70E-06	0.000228
3_20733	3.858477	8.21319	1.88E-05	0.000469
1_1801	4.018757	4.70903	2.88E-05	0.001337
2_14427	4.124717	3.09713	5.16E-05	0.002273
25a-mi-122	-1.74149	16.65976	5.34E-45	0.000007
15_3007	2.250412	8.18102	9.30E-05	0.002067
3_26819	3.786104	2.869811	0.000185	0.008935
18_14354	2.63913	5.04481	0.001163	0.008935
1_1878	-0.5348	2.844225	0.00217	0.007292
1_1796	-3.03205	4.72772	0.000229	0.007345
2_14968	-1.2632	2.241995	0.00032	0.008088
18_12384	1.644726	7.18715	0.00032	0.008088
6_36316	2.706186	4.391608	0.000387	0.0112
3_25735	1.873312	8.891	0.000429	0.011641
2_10588	2.009669	5.882702	0.00054	0.01411
14_7220	-2.8781	5.14484	0.000625	0.015728
20_16918	1.522508	8.095078	0.000716	0.017257
3_26896	3.889885	2.872591	0.000734	0.017257
18_12949	2.884247	3.121942	0.000813	0.01848
20_18738	3.259907	2.783159	0.00097	0.021363
18_10366	3.881196	4.09216	0.001144	0.0241
25a-mi-30a	-1.28354	13.50885	6.001182	6.8241
3_28820	-8.26877	3.148373	0.001246	0.02513
3_30882	3.000233	5.502319	0.001426	0.027871
19_13771	3.264343	4.099403	0.001452	0.027871
6_36380	7.529427	2.373885	0.001886	0.030855
21_17175	4.187755	2.831138	0.001897	0.030855
25a-mi-430b	-1.62115	4.833285	8.001854	8.82237
11_8108	1.887434	5.911269	0.001884	0.032381
12_10861	3.887688	2.526514	0.002027	0.033035
21_17251	1.906177	3.656591	0.002455	0.040285
25a-mi-130b	1.182457	9.071891	8.002534	8.848441
18_12906	1.706743	5.240962	0.002703	0.042344
3_37514	7.147023	2.232375	0.002944	0.043425
17_11580	3.155545	4.528578	0.003205	0.046596
10_2018	1.487722	5.019623	0.003587	0.048596
25a-mi-143a	-1.05942	14.31247	8.002621	8.848441
25a-mi-143b	-1.05942	14.31247	8.002622	8.848441
20_16810	2.835206	3.249947	0.003666	0.048596
3_30762	2.407363	3.372239	0.003697	0.048596
25a-mi-435	-1.62115	9.00065	8.00375	8.848441
15_30027	-2.17853	2.292443	0.003789	0.048596
25a-mi-192	-1.03871	15.03332	0.004548	0.051881
6_36692	2.720892	2.747582	0.004709	0.05836
25a-mi-34c	2.638776	5.438427	0.004716	0.05836
12_10307	1.75594	4.795466	0.004815	0.05836

**Validation and relative
quantification of selected
targets via RT-PCR**



Change of strategy: working on the exosomal part of the milk

UPDATES

- 💧 Strategy: miRNAome of exosome fraction
- 💧 Samples are at the platform lab (Turin)
- 💧 The exosomes have been purified and miRNA have been extracted
- 💧 Sequencing is ongoing

University of Turin

**Raffaele Calogero
Maddalena Arigoni**

University of Guelph

Angela Canovas

UMIL TEAM

**Chiara Gini
Fabrizio Ceciliani**

The sequencing pipeline

Each microRNA sequencing project consists of three main parts:



1. Library preparation (labelling each sample)

2. Deep sequencing

3. Data analysis and multi-group comparison

- statistics (which miRNA are differentially expressed)
- bioinformatics (what are the targets of the miRNA)
- validation, on the same and other samples of differently abundant miRNA

~~October 2021?~~

December 2021

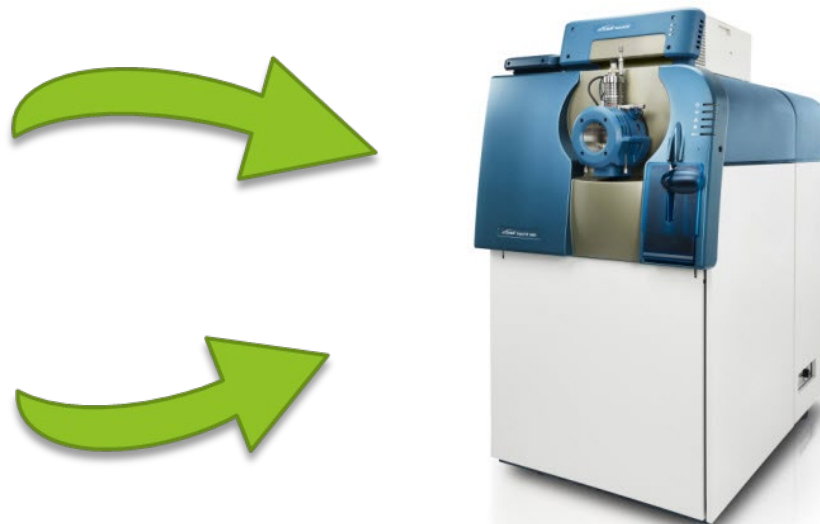


D5.3 A list of milk metabolites involved in the immune response which are modified after different EOs regimen

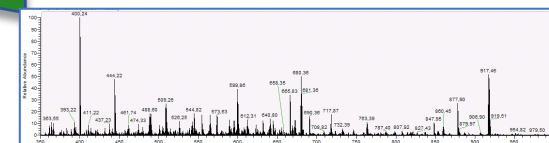
CSIC
Plasma - Task 4.2.1

LPAF (INRAT)
Milk – Task 4.2.2

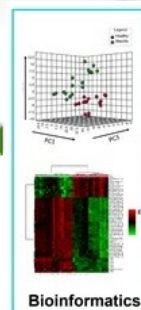
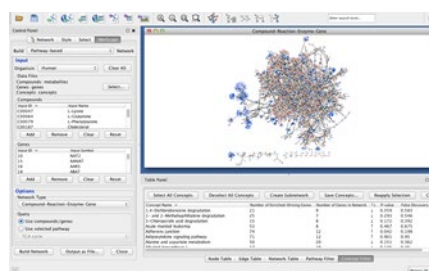
IDELE
Milk – Task 4.4



Metabolome determination
TripleTOF 6600 Plus



Mass spectra



**Identification of metabolites
(Lipids) via bioinformatics**

Triglycerides (TAG, n = 584)
Sphingomyelins (SM, n = 288)
Ceramides (Cer, n = 148)
Ceramides 1-P (CerP, n = 19)
Hexosylceramides (HexCer, n = 145)
Diglycerides (DAG, n = 271)
Phosphatidylcholines (PC, n = 92)
Lysophosphatidylcholines (LPC, n = 22)
Lysophosphoethanolamine (LPE, n = 53)
Phosphatidylinositol (PI, n = 46)
Phosphatidylserine (PS, n = 34)
Monoglycerides (MAG, n = 8)
Cholesterol esters (CE, n = 47)
Fatty acids (FA, n = 97)
Unknown (n = 164)
Other (n = 3)

Lipids classified in 16 classes

(Partial) changes of plan: milk lipidomics and plasma metabolomics

UPDATES

UMIL TEAM

- ✓ 1. Extraction of lipids (whole milk) – one week
- ✓ 2. Separation via HPLC and mass spectrometry – one day
- ✓ 3. Identification of each single lipid (2/3 months) (2400 lipids detected)

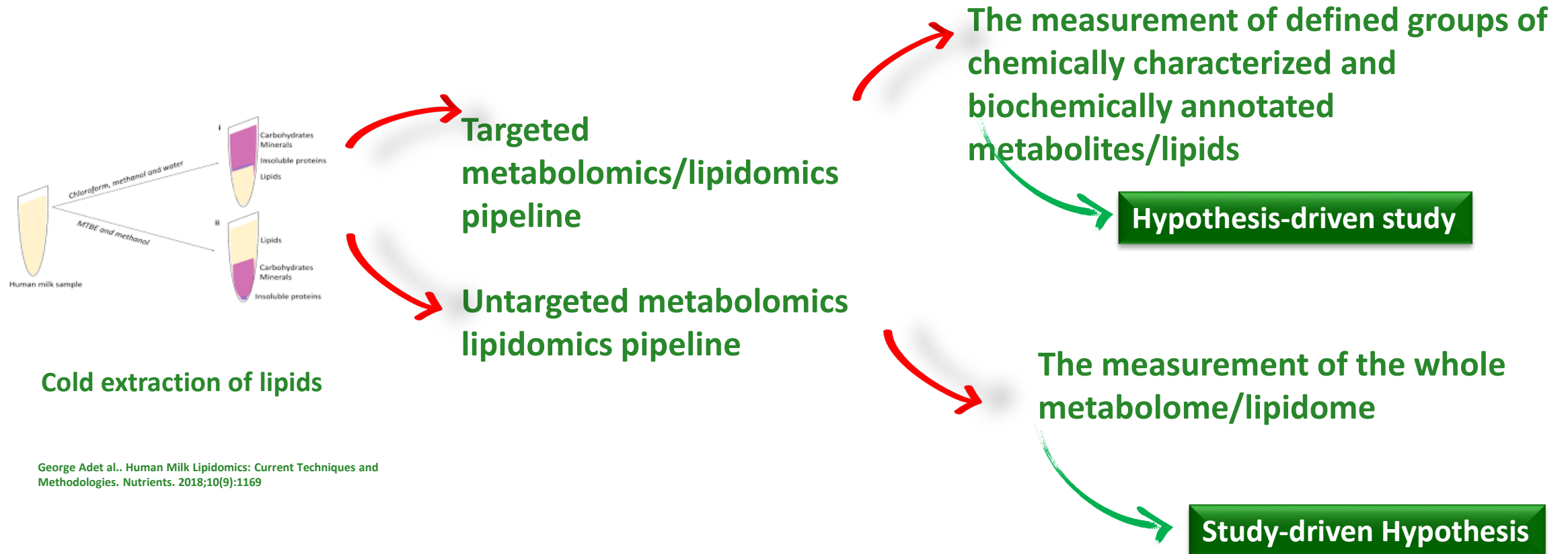
4. *Statistics and bioinformatics: Data analysis and multi-group comparison (NOT READY!!)*

Donatella Caruso
Fiorenza Farè
Manuela Fontana
Chiara Gini
Fabrizio Ceciliani

~~November 2021?~~

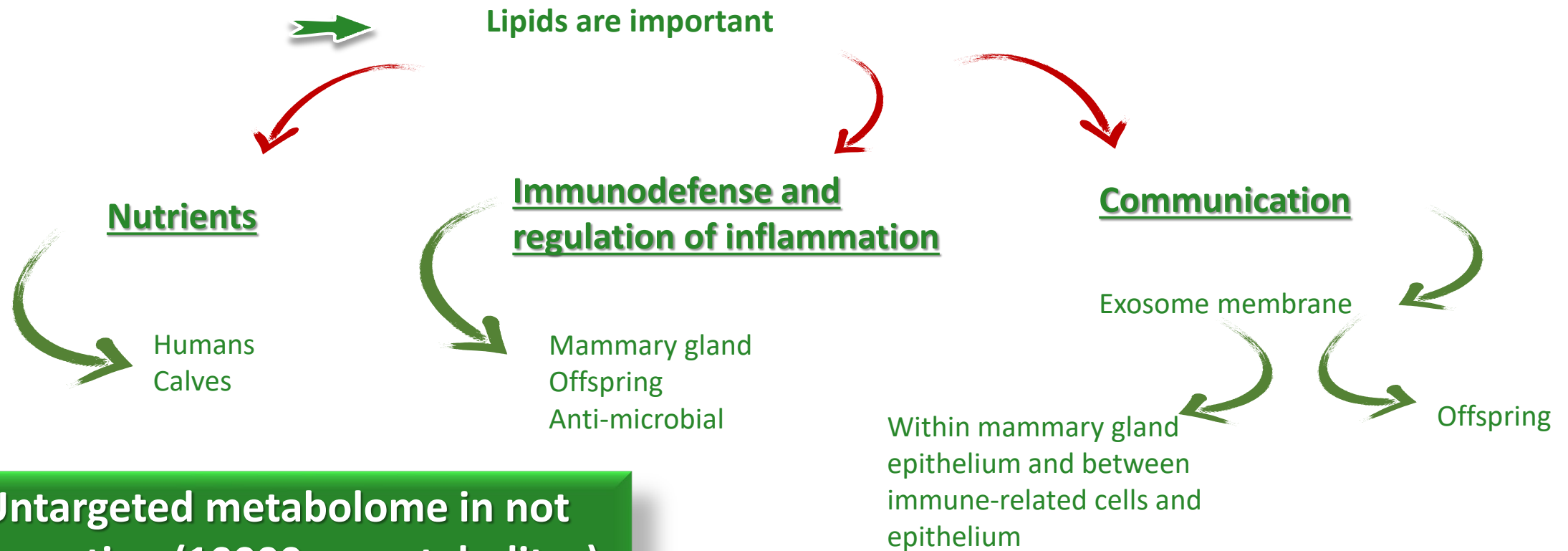
September 2021

The untargeted/targeted metabolomics option



Why we moved to lipidomics?

➔ Lipidome is poorly known



Untargeted metabolome is not an option (10000++ metabolites)



Lipidomics (preliminary) results: the output

Lipid name	Control								Treated								ttest	
	Group	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T		
	Sample	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T		
	ID	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T		
	Sample Name	t28_C1	t28_C2	t28_C3	t28_C4	t28_C5	t28_C6	t28_C7	t28_C8	t28_T1	t28_T2	t28_T3	t28_T4	t28_T5	t28_T6	t28_T7	t28_T8	
NAE 12:0	244,2179	3975,55	4943,36	3614,43	2519,85	5762,3	5083,04	4728,66	5150,35	5049,61	6203,03	6585,04	6556,56	14216,73	7199,24	5546,67	5822,09	0,029106
MG 15:0	334,3066	578,52	478,03	214,59	716,08	555,77	604,54	657,81	446,39	813,63	742,77	1487,91	1527,59	1723,03	1093,05	366,51	955,74	0,005938
CAR 13:0	358,3067	682,06	568,71	291,4	848,33	648,3	707,87	780,1	512,39	948,99	820,69	1653,71	1628,24	1692,01	1118,34	485,79	1002,61	0,006543
PC 31:0 PC 15:0_16:0	720,5483	15861,77	16815,45	18034,84	36798,19	30023,85	26554,36	17695,51	35456,96	15125,87	17700,56	19329,32	15374,4	15197,84	20600,56	18529,27	16852,62	0,036018
SM 32:1;2O	733,5472	315,67	277,17	316,28	749,55	489,86	309,37	268,74	328,15	348,18	142,03	389,44	249,87	90,44	202,17	152,04	278,04	0,045858
SM 39:1;3O	789,6534	4299,67	4963,5	5314,37	10523,89	5643,88	3737,51	3904,54	4811,84	3063,56	3562,18	5046,34	2376,87	3403,29	3055,73	2016,57	4371,46	0,030087
SHexCer 35:0;3O	810,5348	1359,2	1955,28	1323,39	6016,12	2303,56	1203,49	1919,31	1945,69	958,51	1007,21	1487,66	1501,68	645,61	1015,53	1048,9	1083,5	0,058693
SHexCer 38:2;2O	832,5809	303,66	405,8	197,45	367,68	326,48	189,36	170,16	117,19	153,54	76,4	230,46	229,36	92,7	145,97	234,25	109,52	0,036435
TG O-52:7 TG O-18:4_16:0_18:3	857,6993	1909	943,63	629,25	1586,77	1106,74	911,86	603,19	642,32	628,17	374,24	1239,32	433,83	632,55	554,5	340,23	455,11	0,035374

Pairwise comparison (preliminary)

- 💧 T0 Control vs T0 treated: are there any differences between the two groups?
- 💧 T28 Control vs T28 treated: are there any changes between Control and Treated after 28 days?

Lipidomics results T0 Control vs T0 treated

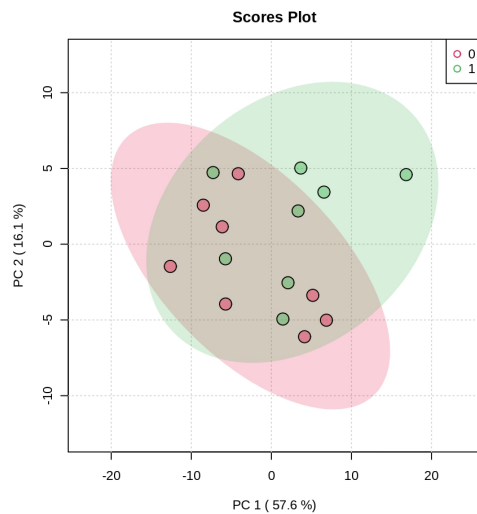
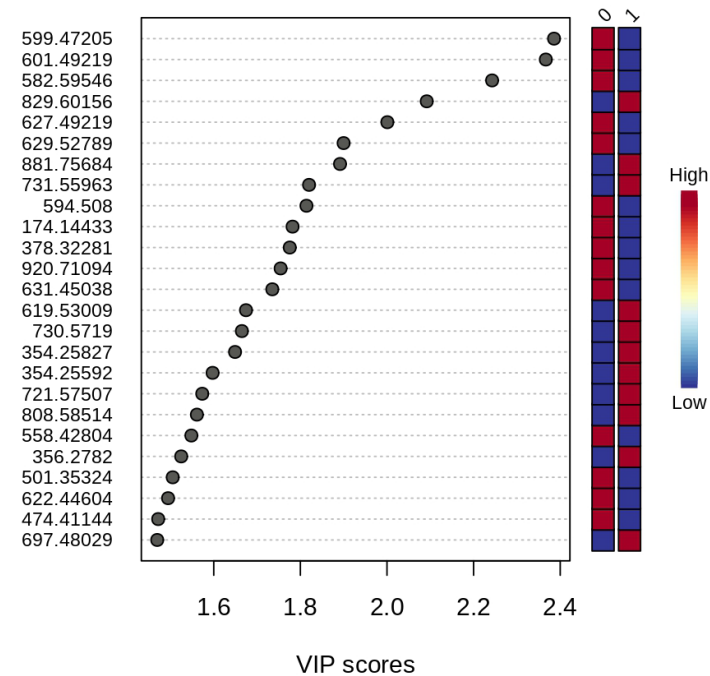


Figure 7: Scores plot between the selected PCs. The explained variances are shown in brackets.

No differences
between T0-C vs T0-T
(except three quarters)

Diacyl Glycerol 33:3



Lipidomics results T28 Control vs T28 treated

Control

Treated

Lipid name	Group	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	ttest
	Sample																	
	ID	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_C	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	t28_T	
	Sample Name	t28_C1	t28_C2	t28_C3	t28_C4	t28_C5	t28_C6	t28_C7	t28_C8	t28_T1	t28_T2	t28_T3	t28_T4	t28_T5	t28_T6	t28_T7	t28_T8	
NAE 12:0	244,2179	3975,55	4943,36	3614,43	2519,85	5762,3	5083,04	4728,66	5150,35	5049,61	6203,03	6585,04	6556,56	14216,73	7199,24	5546,67	5822,09	0,029106
MG 15:0	334,3066	578,52	478,03	214,59	716,08	555,77	604,54	657,81	446,39	813,63	742,77	1487,91	1527,59	1723,03	1093,05	366,51	955,74	0,005938
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SM 39:1;3O	789,6534	4299,67	4963,5	5314,37	10523,89	5643,88	3737,51	3904,54	4811,84	3063,56	3562,18	5046,34	2376,87	3403,29	3055,73	2016,57	4371,46	0,030087
SHexCer 35:0;3O	810,5348	1359,2	1955,28	1323,39	6016,12	2303,56	1203,49	1919,31	1945,69	958,51	1007,21	1487,66	1501,68	645,61	1015,53	1048,9	1083,5	0,058693
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TG O-52:7 TG O-18:4_16:0_18:3	857,6993	1909	943,63	629,25	1586,77	1106,74	911,86	603,19	642,32	628,17	374,24	1239,32	433,83	632,55	554,5	340,23	455,11	0,035374

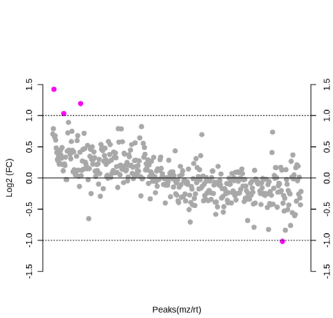


Figure 2: Important features selected by fold-change analysis with threshold 2. The red circles represent features above the threshold. Note the values are on log scale, so that both up-regulated and down-regulated features can be plotted in a symmetrical way

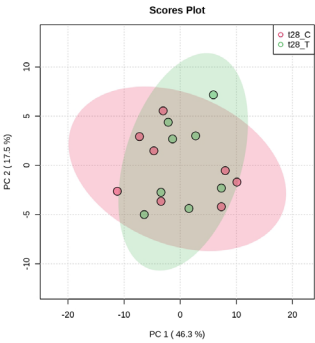


Figure 5: Scores plot between the selected PCs. The explained variances are shown in brackets.

MG 15:0
CAR 13:0

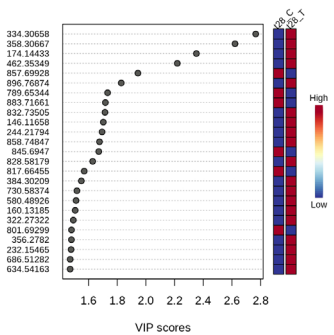


Figure 14: Important features identified by PLS-DA. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group under study.

Plasma metabolomics

- 💧 Will be carried out following a different strategy (Biocrates profiling)
- 💧 Partnership to be decided jointly with UNIMI platform
- 💧 Metabolomics on plasma will be carried out using a targeted approach



D5.4 A list of differentially abundant proteins after feeding with different EOs

Change of strategy: working on the exosomal part of the milk

UPDATES

- 💧 Samples are at the UMIL lab
- 💧 UNIMI platforms have been engaged
- 💧 Strategy: purification of exosomes –UNIBONN/ proteomics - UNIMI

UMIL TEAM

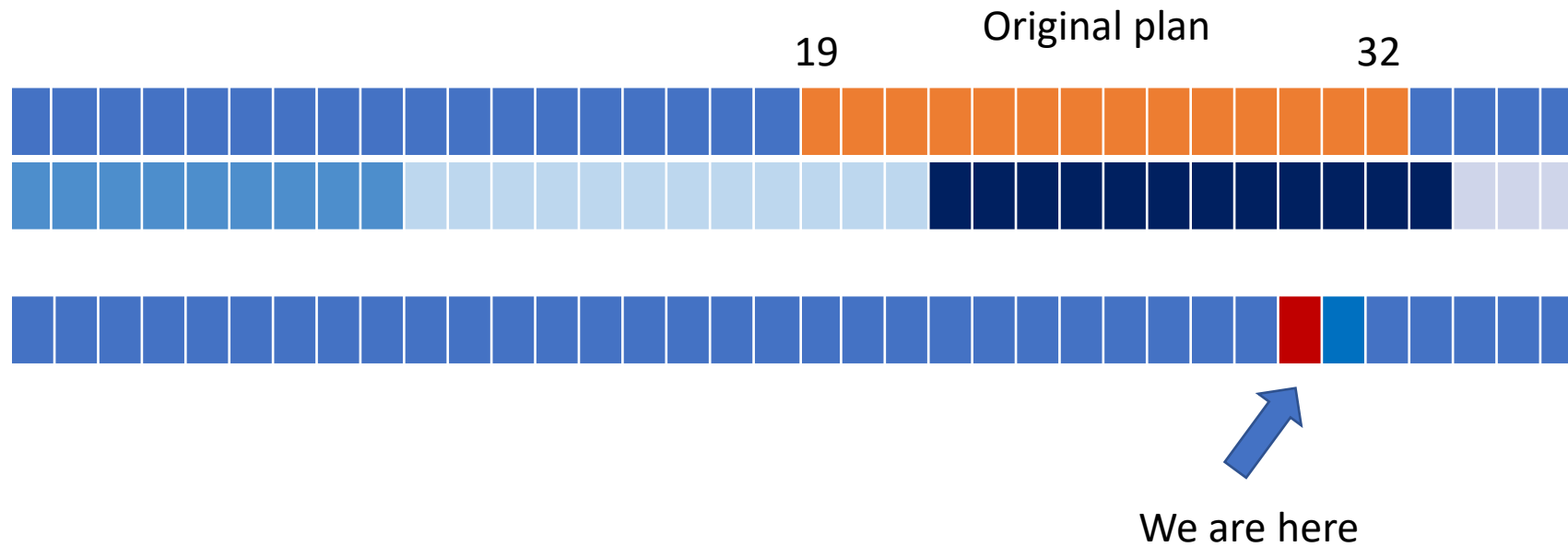
Chiara Gini
Fabrizio Ceciliani
Donatella Caruso

UNIBONN TEAM

Helga Sauerwein

December/January 2021/2022

Time table



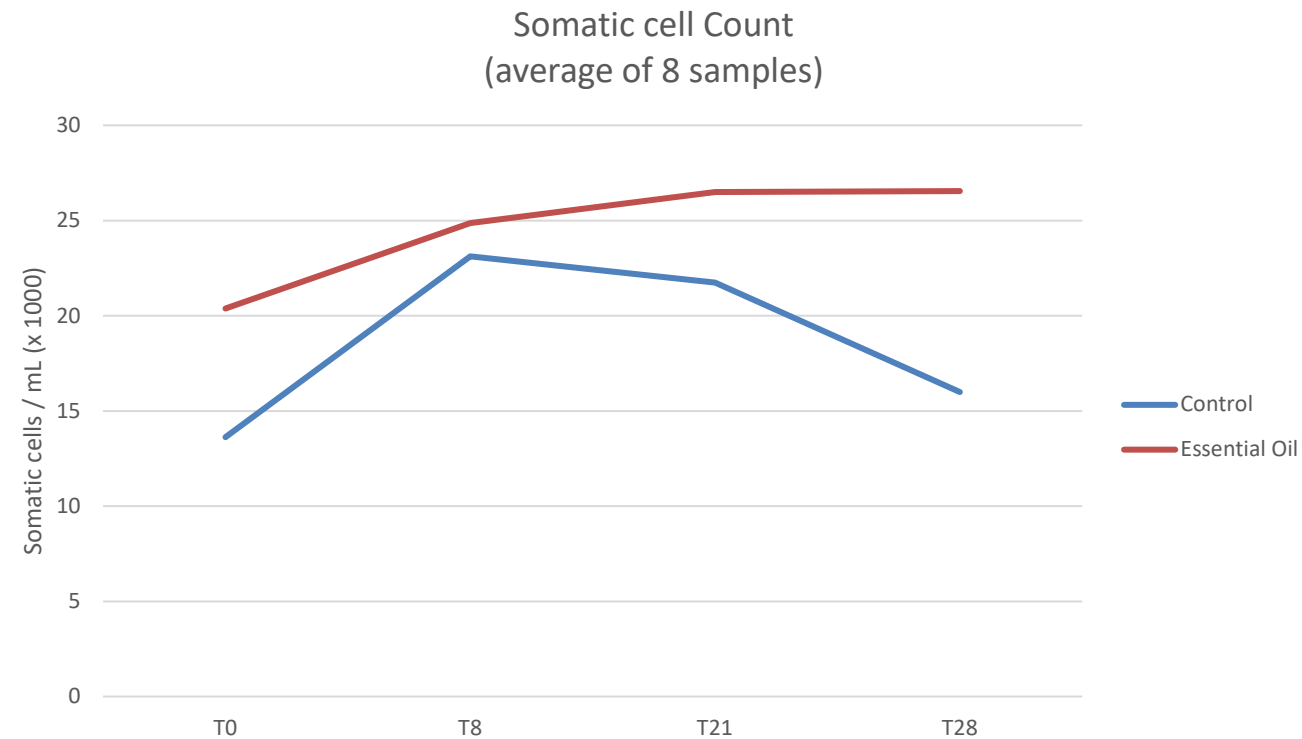
Valorisation

- 💧 Meetings. Non congress in view (to my knowledge) but we are totally open to suggestions
- 💧 Manuscripts: likely the one of lipidomics might be ready soon (months)
- 💧 Manuscripts: Microbiomics: the second to come
- 💧 Others???

Other possible analysis on milk

- 💧 Acute Phase proteins (SAA, Hp)
- 💧 Other inflammatory parameters (lactoferrin)
- 💧 Other suggestions?

Somatic Cell count average quarter milk



It seems so far that EO topic treatment gave no results on the main inflammatory parameter

Thanks for listening