

1 **Supplementing conjugated and non-conjugated L-methionine and acetate alters**
2 **expression patterns of β -casein, proteins, and metabolites related to protein synthesis in**
3 **bovine mammary cells**

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5 **Seungwoo Jeon, Jay Ronel Conejos , Jungeun Kim, Minjeong Kim, Jeongeun Lee,**
6 **Baekseok Lee, Jinseung Park, Junok Moon, Jaesung Lee and Honggu Lee**

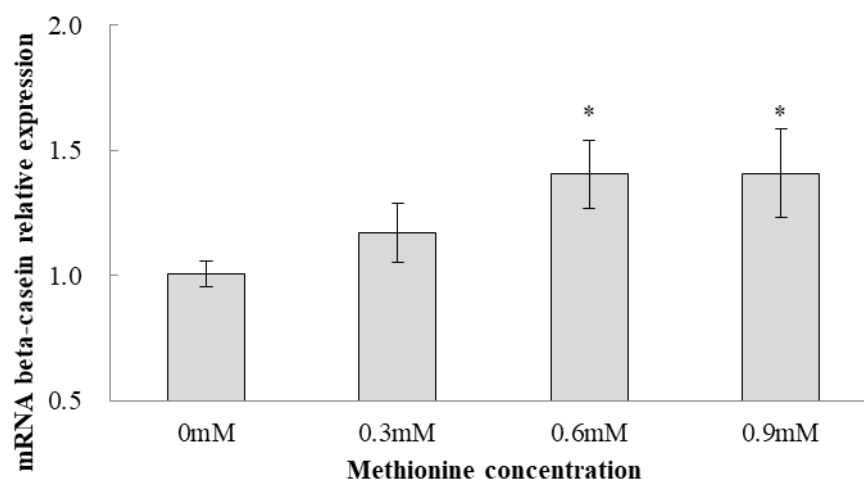
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9 **SUPPLEMENTARY FILE**

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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)



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12 Supplemental Figure 1. β -casein mRNA expression level in MAC-T cells incubated with different
13 levels of L-methionine (0, 0.3, 0.6, 0.9 mM) for 24 h. Values are expressed as means \pm SE (n = 6
14 per group). Letters * indicate significant differences at P < 0.05 by student t-test comparing control
15 (0 mM)

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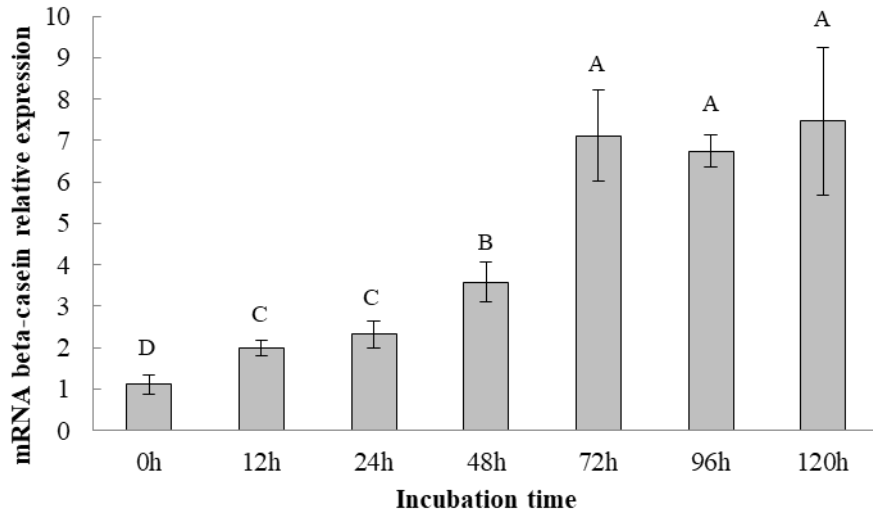
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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

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29 Supplemental Figure 2. β -casein mRNA expression level in MAC-T cells incubated with 0 mM L-
30 methionine at different time points (0, 24, 48, 72, 96, 120 h). Values are expressed as means \pm SE (n
31 = 6 per group) and A, B, C, D indicate significant differences at P < 0.05 by Duncan Multiple
32 Range Test.

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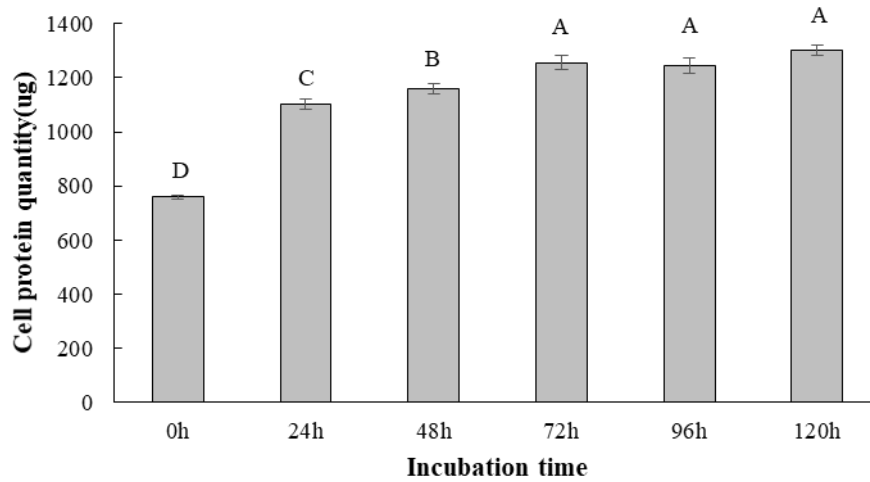
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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

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45 Supplemental Figure 3. Cell protein quantity in MAC-T cells incubated with 0 mM L-methionine at
46 different time points (0, 24, 48, 72, 96, 120 h). Values are expressed as means \pm SE (n = 6 per group)
47 and A, B, C indicate significant differences at $P < 0.05$ by Duncan Multiple Range Test.

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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

51 Supplemental Table 1. List of protein pathways related to protein and energy metabolism
 52 affected by supplementation (in comparison to Control)

Detected Pathway	L-Met ¹	CMA ²	NMA ³
Apoptosis signaling		●	
ATP synthesis	●		●
Cell cycle			●
Dopamine receptor mediated signaling pathway		●	
FAS signaling		●	●
Glycolysis		●	●
Hypoxia response via HIF activation		●	
Nicotine pharmacodynamics		●	
Oxidative stress response		●	
Pentose phosphate		●	
PI3 kinase	●	●	
Purine metabolism		●	
Pyruvate metabolism	●	●	
Ubiquitin proteasome			●
TGF- β signaling			●

53 Pathway analysis (*Bos taurus*). The analysis used increasing detected pathway at $P < 0.05$.

54 ¹L-Met = L-methionine

55 ²CMA = conjugated L-methionine and acetate

56 ³NMA = non-conjugated L-methionine and acetate.

SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

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58 Supplemental Table 2. List of all protein pathways affected by supplementation (in comparison to control)

Detected Pathway	L-Met ¹	CMA ²	NMA ³
5HT2 type receptor mediated signaling	●	●	●
Alzheimer's disease-presenilin	●	●	●
Apoptosis signaling		●	
ATP synthesis	●		●
Cadherin signaling	●	●	●
CCKR signaling map	●	●	●
Cell cycle			●
Cholesterol biosynthesis	●	●	●
Cytoskeletal regulation by Rho GTPase	●	●	●
De novo purine biosynthesis	●	●	●
De novo pyrimidine deoxyribonucleotide biosynthesis	●	●	●
De novo pyrimidine ribonucleotides biosynthesis	●	●	●
Dopamine receptor mediated signaling pathway		●	
EGF receptor signaling	●	●	●
FAS signaling		●	●
FGF signaling	●	●	●
Fructose galactose metabolism			
Glycolysis		●	●
Gonadotropin-releasing hormone receptor	●	●	●

SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

HeterotrimericG-protein signaling pathway -Gi alpha and Gs alpha mediated	●	●	●
Heterotrimeric G-protein signaling pathway -Gq alpha and Go alpha mediated	●	●	●
Histamine H1 receptor mediated signaling	●	●	●
Huntington's disease	●	●	●
Hypoxia response via HIF activation		●	
Inflammation mediated by chemokine and cytokine signaling	●	●	●
Integrin signaling	●	●	●
Nicotine pharmacodynamics		●	
Nicotinic acetylcholine receptor signaling	●	●	●
Oxidative stress response		●	
Oxytocin receptor mediated signaling	●	●	●
p53	●	●	●
Parkinson's disease	●	●	●
Pentose phosphate		●	
PI3 kinase	●	●	
Purine metabolism		●	
Pyruvate metabolism	●	●	
Salvage pyrimidine ribonucleotides	●	●	●
Thyrotropin-releasing hormone receptor signaling	●	●	●

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Wnt signaling	●	●	●
Ubiquitin proteasome			●
TGF- β signaling			●

59 Pathway analysis (*Bos taurus*). The analysis used increasing detected pathway at $P < 0.05$.

60 ¹L-Met = L-methionine

61 ²CMA = conjugated L-methionine and acetate

62 ³NMA = non-conjugated L-methionine and acetate.

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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

77 Supplemental Table 3. List of differently expressed proteins in MAC-T Cells (in comparison to L-Met)

Detection Protein	CMA¹	NMA²
Increasing Number	67	37
Decreasing Number	47	40
Total Protein Number	114	77
List of Selected Downregulated and Upregulated Proteins		
Eukaryotic Translation Initiation Factor 3 Subunit A (EIF3A)		▲
Ribosomal Protein S21(RPS21)		▼
Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)	▲	▼
Eukaryotic Translation Initiation Factor 4A1 (EIF4A1)	▼	
EIF4A2 (Eukaryotic Translation Initiation Factor 4A2)	▲	
ATP Synthase, H ⁺ Transporting, Mitochondrial F1 Complex, Alpha Subunit 1 (ATP5A1)	▲	▲
Ribosomal Protein S12 (RPS21)		▼
Elongation factor 1-alpha 1 (EEF1A1)		▲
ATP synthase subunit β, mitochondrial (ATP5B)	▼	

78 Selection Criteria: Upregulated, > 2-fold in protein expression vs. L-Met,

79 Downregulated: < 0.5-fold in protein expression vs. L-Met.

80 ¹CMA = conjugated L-methionine and acetate

81 ²NMA = non-conjugated L-methionine and acetate.

SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

82 Supplemental Table 4. List of all protein pathways affected by treatment supplementation (L-
83 Met as control).

Detected Pathway	CMA ¹	NMA ²	Both
5HT2 type receptor mediated signaling pathway		●	
ALP23B_signaling_pathway		●	
Apoptosis signaling pathway	●	●	
Alzheimer disease-presenilin pathway		●	
ATP synthesis	●	●	
BMP_signaling_pathway-drosophila		●	
Cadherin signaling pathway	●	●	
Cholesterol biosynthesis		●	
Cytoskeletal regulation by Rho GTPase	●	●	
De novo purine biosynthesis	●		
De novo pyrimidine deoxyribonucleotide biosynthesis		●	
De novo pyrimidine ribonucleotides biosynthesis		●	
EGF receptor signaling pathway	●	●	●
FAS signaling pathway	●		
FGF signaling pathway		●	●
Glycolysis		●	
Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway	●		
Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	●		

SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

Histamine H1 receptor mediated signaling pathway		●	
Huntington disease	●	●	
Hypoxia response via HIF activation			
Inflammation mediated by chemokine and cytokine signaling pathway	●	●	
Integrin signaling pathway	●	●	●
MYO signaling pathway		●	
Nicotinic acetylcholine receptor signaling pathway	●	●	
Nicotine pharmacodynamics pathway	●		
Oxytocin receptor mediated signaling pathway		●	
p53 pathway	●	●	●
Parkinson disease	●	●	●
Pentose phosphate pathway	●		
TGF- β signaling pathway		●	
Thyrotropin-releasing hormone receptor signaling pathway		●	
Wnt signaling pathway		●	

84 Pathway analysis (Bos taurus). The analysis used increasing detected pathway at $P < 0.05$.

85 ¹CMA = conjugated L-methionine and acetate

86 ²NMA = non-conjugated L-methionine and acetate.

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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

90 Supplemental Table 5. List of differently expressed proteins in MAC-T Cells (in comparison
91 to NMA²)

Detection Protein	CMA¹
Increasing Number	75
Decreasing Number	52
Total Protein Number	127
List of Selected Downregulated and Upregulated Proteins	
Eukaryotic Translation Initiation Factor 3 Subunit A (EIF3A)	▲
Ribosomal Protein S21(RPS21)	▲
Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)	▲
Eukaryotic Translation Initiation Factor 4A1 (EIF4A1)	▼
Eukaryotic Translation Initiation Factor 4A2 (EIF4A2)	▲
Ribosomal Protein S12 (RPS21)	▲
Elongation factor 1-alpha 1 (EEF1A1)	▼
ATP synthase subunit β , mitochondrial (ATP5B)	▼
FASN Fatty acid synthase	▼

92 Selection Criteria: Upregulated, > 2-fold in protein expression vs. NMA,

93 Downregulated: < 0.5-fold in protein expression vs. NMA.

94 ¹CMA = conjugated L-methionine and acetate

95 ²NMA = non-conjugated L-methionine and acetate.

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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

97 Supplemental Table 6. List of all protein pathways affected by treatment supplementation
 98 (NMA² as control).

Detected Pathway	CMA¹
Apoptosis signaling pathway	●
Huntington disease	●
Integrin signaling pathway	●
De novo pyrimidine deoxyribonucleotide biosynthesis	●
Parkinson disease	●
FAS signaling pathway	●
Alzheimer disease-presenilin pathway	●
Nicotine pharmacodynamics pathway	●
PI3 kinase pathway	●
Cytoskeletal regulation by Rho GTPase	●
Nicotinic acetylcholine receptor signaling pathway	●
De novo purine biosynthesis	●
Pentose phosphate pathway	●
Inflammation mediated by chemokine and cytokine signaling pathway	●
Wnt signaling pathway	●
EGF receptor signaling pathway	●
Cadherin signaling pathway	●
Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway	●

SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway 2	●
Salvage pyrimidine ribonucleotides	●
De novo pyrimidine ribonucleotides biosynthesis	●

99 Pathway analysis (Bos taurus). The analysis used increasing detected pathway at $P < 0.05$.

100 ¹CMA = conjugated L-methionine and acetate

101 ²NMA = non-conjugated L-methionine and acetate.

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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

118 Supplemental Table 7. List of Pathway Related to Detection Metabolite

Metabolite related pathway	L-Met ¹	CMA ²	NMA ³
Alanine, aspartate and glutamate metabolism	●	●	
Aminoacyl-tRNA biosynthesis	●	●	●
Arginine and proline metabolism	●	●	●
β-alanine metabolism	●	●	
Butanoate metabolism	●		
Citrate cycle (TCA cycle)	●	●	●
Cysteine and Methionine metabolism	●	●	●
D-glutamine and D-glutamate metabolism	●		
Glycine, serine and threonine metabolism	●		
Glycolysis or Gluconeogenesis	●		
Glyoxylate and dicarboxylate metabolism	●	●	●
Histidine metabolism	●	●	
Inositol phosphate metabolism			●
Pantothenate and CoA biosynthesis		●	●
Pentose phosphate pathway			●
Phenylalanine metabolism	●	●	●
Phenylalanine, tyrosine and tryptophan biosynthesis	●	●	●
Propanoate metabolism		●	●
Pyruvate metabolism	●	●	●

SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

Tyrosine metabolism	●	●	●
Ubiquinone and other terpenoid-quinone biosynthesis	●	●	●
Valine, leucine and isoleucine biosynthesis	●	●	●
Valine, leucine and isoleucine degradation	●	●	●

119 Metabo Analyst 3.0 was used to perform the pathway analysis (*Bos taurus*). The analysis used increasing
120 detection metabolite at $P < 0.05$.

121 ¹L-Met = L-methionine

122 ²CMA = conjugated L-methionine and acetate

123 ³NMA = non-conjugated L-methionine and acetate.

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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

138 Supplemental Table 8. List of Detected Metabolite affected by application of treatment

Category	L-Met¹	CMA²	NMA³
Increasing Metabolite List	Pyruvate	NADH	Phenylalanine
	2-Oxoglutarate	Valine	Glucose 6-phosphate
	Fumarate	Aspartate	Fructose 6-phosphate
	N-acetyl-L-glutamate	Phenylalanine	Xylulose5-P
	Malate	Xylulose5-P	2-oxoisovalerate
	Aspartate	2-oxoisovalerate	Malate
	Met	Malate	Met
	Leu	Met	Leu
	Tyrosine	Leu	Tyrosine
	Ile	Tyrosine	N-acetyl-L-glutamate
		6-Phosphogluconate	Glucose 1-phosphate
		Ile	6-Phosphogluconate
Decreasing Metabolite List		UMP	IMP
			Pantothenate

139 The Detected Metabolite was selected by Student's T-test (Control vs. Treatment, $P < 0.05$).

140 ¹L-Met = L-methionine

141 ²CMA = conjugated L-methionine and acetate

142 ³NMA = non-conjugated L-methionine and acetate.

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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

148 Supplemental Table 9. List of Detected Metabolite using L-Met as control.

Category	CMA¹	NMA²
Increasing Metabolite List	Alanine	Valine
	Glutamine	Methionine
	2-oxoisovalerate	Isoleucine
	Fructose 1,6-bisphosphatase	Leucine
	NADH	Tyrosine
	FAD	Phenylalanine
		Glucose-6-Phosphate
		Fructose-6-Phosphate
		Fructose-1-Phosphate
		2-oxoisovalerate
	Acetate-Glutamate	
	Aspartate	
Decreasing Metabolite List	Methionine	
	UMP	Glycerol-3-Phosphate
	IMP	Pyruvate
	AMP	NAD ⁺
	Acetate-Glutamate	UMP
	Fumarate	IMP
	Phosphoenolpyruvate	GMP
	UDP-glucuronic acid	Pantothenate
		AMP
		6-Phosphoglycerate
		Citrate
		Guanosine diphosphate
		NADPH

149 The Detected Metabolite was selected by Student's T-test (L-Met vs. Treatment, P < 0.05).

150 ¹CMA = conjugated L-methionine and acetate

151 ²NMA = non-conjugated L-methionine and acetate.

SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

152 Supplemental Table 10. List of Detected Metabolite (NMA² as control).

Category	CMA¹	p-value
Decrease Metabolite List	Methionine	<0.0001
	Isoleucine	0.046
	Phenylalanine	0.002
	Glucose-6-Phosphate	0.002
	Fructose-6-Phosphate	0.001
	Glucose-1-Phosphate	0.008
Increase Metabolite List	Acetate-Glutamate	<0.0001
	6-Phosphoglycerate	0.012
	Serine	0.046
	Aspartate	0.020
	Glycerol-3-Phosphate	0.044
	NAD ⁺	0.007
	Dihydroxyacetone phosphate	0.033
	Inosinic acid	0.002
	Pantothenate	0.042
	Glycerate-3-Phosphate	0.012
	Fructose 1,6-bisphosphatase	0.022
	Citrate	0.029
	NADP	0.048
	Guanosine diphosphate	0.062
	Phosphoenol pyruvate	0.085
	NADH	0.024
UDp-glucuronic acid	0.038	
NADPH	0.046	

153 The Detected Metabolite was selected by Student's T-test (NMA vs. Treatment, P < 0.05).

154 ¹CMA = conjugated L-methionine and acetate

155 ²NMA =

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SUPPLEMENTING CONJUGATED L-METHIONINE AND ACETATE (CMA)

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