

1 **Immune reactivity against a variety of mammalian and plant-based milks:**

2 **Supplemental Files**

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35 **Materials & Methods**

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*Inhibition of cow's milk antibodies by different milks.*

Affinity-purified human IgE and IgG against cow's milk were used for inhibition studies with different mammalian and plant milks.

For the inhibition of cow's milk IgE antibodies binding to cow's milk-coated plates by different milks, five different rows of microtiter plates were coated with 10 µg of cow's milk antigens per well. 100 µl of serum diluents was added to wells A1-E2 and 100 µl of serially diluted (400 µg/mL) cow's milk, goat's milk, camel milk, human milk and coconut milk to wells A2-A8, B2-B8, C2-C8, D2-D8 and E2-E8 respectively. Plates were incubated for 1 hr at 37°C and 100 µl of affinity purified anti-cow's milk IgE was added to all wells. After repeated incubation and washing 100 µl of alkaline phosphatase labeled Anti-IgE antibody was added to all wells, then the plate was incubated 1hr at 24°C. After washing and addition of substrate the ODs were recorded.

For the inhibition of anti-cow's milk IgG antibody binding to cow's milk-coated plates by different milks, all steps were similar to IgE inhibition, with the exception of using almond milk instead of coconut as control, and addition of anti-human IgG instead of anti-IgE.

69 **Results**

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71 *Immune reactions to different cow's milks.*

72 **Table S1.** Measurement of IgE antibody against different types of cow's milk in 24  
 73 individuals, some with low and some with high antibody levels against regular cow's milk,  
 74 shown as ELISA index. ELISA index greater than 1.4 is considered significant.

<b>IgE</b>	<b>Regular Milk</b>	<b>Organic Milk</b>	<b>Raw Jersey Grass-Fed</b>	<b>Raw Holstein Milk A1</b>	<b>Raw Holstein Milk A2</b>
<b>1</b>	2.4	2.3	1.8	1.9	2.1
<b>2</b>	0.81	0.74	0.93	0.66	0.75
<b>3</b>	0.15	0.17	0.10	0.11	0.18
<b>4</b>	2.8	2.3	2.9	2.6	1.8
<b>5</b>	1.5	1.1	1.6	1.8	1.5
<b>6</b>	0.51	0.33	0.45	0.49	0.58
<b>7</b>	2.1	2.5	2.2	2.7	1.9
<b>8</b>	0.82	0.97	0.85	1.0	1.3
<b>9</b>	0.13	0.23	0.21	0.11	0.14
<b>10</b>	2.6	2.9	3.3	2.7	3.4
<b>11</b>	1.3	1.1	1.6	1.6	1.1
<b>12</b>	0.24	0.18	0.32	0.29	0.24
<b>13</b>	1.96	0.52	0.57	0.73	0.79
<b>14</b>	1.8	1.5	2.1	1.9	1.6
<b>15</b>	0.84	1.3	1.1	1.2	1.5
<b>16</b>	0.35	0.37	0.54	0.44	0.36
<b>17</b>	1.8	2.2	1.9	2.1	1.6
<b>18</b>	1.5	1.4	1.7	1.2	1.0
<b>19</b>	0.51	0.83	0.54	0.42	0.48
<b>20</b>	0.24	0.30	0.31	0.36	0.32
<b>21</b>	2.7	3.3	2.6	1.7	1.8
<b>22</b>	1.8	1.5	1.3	0.97	1.3
<b>23</b>	0.39	0.43	0.52	0.46	0.42
<b>24</b>	3.4	2.9	2.6	2.9	2.4

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79 **Table S2.** Measurement of IgG antibody against different types of cows' milk in 24  
80 individuals, some with low and some with high antibody levels against regular cow's milk,  
81 shown as ELISA index. ELISA index greater than 1.7 is considered significant.

<b>IgG</b>	<b>Regular Milk</b>	<b>Organic Milk</b>	<b>Raw Jersey Grass-Fed</b>	<b>Raw Holstein Milk A1</b>	<b>Raw Holstein Milk A2</b>
<b>1</b>	1.9	1.8	2.0	2.3	2.1
<b>2</b>	0.46	0.49	0.53	0.55	0.53
<b>3</b>	1.9	1.8	2.4	1.6	1.4
<b>4</b>	0.23	0.15	0.26	0.25	0.12
<b>5</b>	2.5	2.1	2.8	2.3	2.5
<b>6</b>	0.22	0.15	0.11	0.13	0.15
<b>7</b>	1.6	1.4	1.6	1.9	1.8
<b>8</b>	1.8	1.6	2.4	2.3	1.7
<b>9</b>	0.51	0.55	0.65	0.83	0.77
<b>10</b>	2.7	1.9	2.7	1.6	1.9
<b>11</b>	1.9	1.8	1.9	1.9	2.6
<b>12</b>	3.1	2.7	3.2	2.8	2.1
<b>13</b>	0.62	0.41	0.38	0.50	0.45
<b>14</b>	1.1	1.0	1.4	1.6	1.2
<b>15</b>	0.43	0.61	0.68	0.55	0.61
<b>16</b>	2.4	2.1	2.4	2.5	2.3
<b>17</b>	0.15	0.13	0.16	0.31	0.22
<b>18</b>	1.4	1.4	2.1	1.6	1.3
<b>19</b>	0.67	0.73	0.81	0.63	0.51
<b>20</b>	1.5	1.3	2.0	1.8	1.6
<b>21</b>	0.8	0.7	1.3	0.98	1.2
<b>22</b>	0.61	0.45	0.33	0.47	0.45
<b>23</b>	0.8	0.7	0.9	1.4	0.95
<b>24</b>	1.3	1.2	2.0	1.8	1.6

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84 **Table S3.** Measurement of IgA antibody against different types of cows' milk in 24  
 85 individuals, some with low and some with high antibody levels against regular cow milk,  
 86 shown as ELISA index. ELISA index greater than 1.6 is considered significant.

<b>IgA</b>	<b>Regular Milk</b>	<b>Organic Milk</b>	<b>Raw Jersey Grass- Fed</b>	<b>Raw HolsteinMilk A1</b>	<b>Raw Holstein Milk A2</b>
<b>1</b>	1.1	0.9	1.1	1.3	1.6
<b>2</b>	0.29	0.52	0.47	0.45	0.50
<b>3</b>	1.1	1.0	0.9	0.9	1.1
<b>4</b>	0.33	0.24	0.17	0.18	2.3
<b>5</b>	0.8	0.75	0.9	0.84	0.92
<b>6</b>	0.38	0.56	0.62	0.6	0.58
<b>7</b>	0.8	0.78	0.8	0.85	0.73
<b>8</b>	0.16	0.21	0.18	0.26	0.22
<b>9</b>	1.3	1.2	1.4	1.4	1.1
<b>10</b>	0.60	0.75	0.67	0.63	0.76
<b>11</b>	1.0	0.8	1.2	1.2	1.4
<b>12</b>	1.1	0.9	0.95	1.3	1.2
<b>13</b>	0.67	0.53	0.74	0.66	0.54
<b>14</b>	1.9	1.8	1.9	1.6	1.8
<b>15</b>	1.1	1.4	1.6	1.4	1.1
<b>16</b>	0.4	0.7	0.9	0.98	1.3
<b>17</b>	0.7	0.6	0.8	0.72	0.84
<b>18</b>	2.3	1.9	2.1	1.8	2.0
<b>19</b>	1.0	1.1	1.4	1.3	1.1
<b>20</b>	1.4	1.5	1.9	1.9	1.6
<b>21</b>	1.8	1.7	1.6	1.4	1.2
<b>22</b>	0.31	0.23	0.15	0.16	0.20
<b>23</b>	1.2	0.8	1.1	1.3	1.0
<b>24</b>	1.7	2.2	2.0	2.2	2.4

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91 *Immune reaction to goat, sheep, camel, human and plant milks.*

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93 **Table S4.** Correlation between the levels of IgE antibodies against different mammalian and  
94 plant milks shown as ELISA index.

<b>IgE</b>	<b>Cow</b>	<b>Goat</b>	<b>Sheep</b>	<b>Camel</b>	<b>Human</b>	<b>Soy</b>	<b>Almond</b>	<b>Coconut</b>
<b>1</b>	2.1	1.6	1.2	1.0	0.6	1.8	2.1	0.6
<b>2</b>	3.6	2.0	1.1	1.1	0.7	1.4	0.8	0.4
<b>3</b>	2.2.	1.2	1.4	1.2	0.9	1.8	2.6	0.8
<b>4</b>	2.8	2.0	2.2	1.4	0.6	1.2	1.0	0.6
<b>5</b>	2.6	1.4	2.0	1.2	0.7	1.4	1.2	0.7
<b>6</b>	2.3	2.6	2.0	1.6	0.8	1.6	0.9	0.9
<b>7</b>	2.4	1.2	1.4	0.8	0.6	0.8	1.6	0.6
<b>8</b>	3.9	3.2	3.6	2.1	2.7	2.4	3.2	1.7
<b>9</b>	2.1	1.4	1.8	1.4	1.1	1.5	3.6	1.3
<b>10</b>	2.2	1.6	1.4	2.2	0.8	1.1	1.0	0.6
<b>11</b>	2.7	2.3	2.2	1.6	0.6	0.8	0.9	0.5
<b>12</b>	3.1	2.2	2.6	1.8	1.5	1.7	1.7	0.9
<b>13</b>	3.2	2.0	2.3	1.8	1.9	2.0	2.1	1.4
<b>14</b>	2.8	2.1	2.4	1.9	2.5	2.4	1.6	1.5
<b>15</b>	2.0	2.0	1.9	3.4	1.4	2.9	3.8	3.0
<b>16</b>	2.4	3.11	3.6	1.2	1.1	1.3	1.8	1.4
<b>17</b>	2.5	2.6	3.4	4.3	5.1	4.2	4.9	3.1
<b>18</b>	2.0	1.5	1.8	2.1	1.4	1.3	2.6	1.6
<b>19</b>	1.8	2.0	2.1	2.2	1.5	1.6	3.8	1.4
<b>20</b>	1.9	1.4	1.2	2.1	0.8	2.3	1.0	1.2
<b>21</b>	2.2	1.6	1.7	1.2	1.4	1.4	1.1	1.1
<b>22</b>	1.8	2.0	1.8	0.9	0.4	2.0	1.6	0.6
<b>23</b>	2.4	3.2	3.2	1.3	1.8	3.4	2.7	1.9
<b>24</b>	1.8	1.7	1.4	1.8	1.8	2.4	7.2	3.7
<b># out of 24</b>	24/24	19/24	17/24	14/24	8/24	15/24	16/24	7/24
<b>% Positive</b>	100%	79%	71%	58%	33%	63%	67%	29%

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97 **Table S5.** Correlation between the levels of IgG antibodies against different mammalian and  
 98 plant milks shown as ELISA index.

<b>IgG</b>	<b>Cow</b>	<b>Goat</b>	<b>Sheep</b>	<b>Camel</b>	<b>Human</b>	<b>Soy</b>	<b>Almond</b>	<b>Coconut</b>
<b>1</b>	3.0	3.2	2.9	2.4	1.2	2.3	2.4	0.9
<b>2</b>	3.2	4.6	3.0	1.8	3.5	2.4	1.3	1.3
<b>3</b>	4.0	3.9	3.1	1.9	2.0	1.6	1.4	1.6
<b>4</b>	3.5	3.6	2.8	1.6	1.1	2.0	1.5	2.1
<b>5</b>	2.9	2.8	2.7	1.4	2.0	3.0	1.9	2.2
<b>6</b>	4.1	4.9	3.3	3.8	4.3	2.1	1.3	1.3
<b>7</b>	4.0	4.8	2.6	1.7	4.0	5.2	1.3	1.2
<b>8</b>	3.9	4.9	3.5	3.3	2.2	3.1	1.2	2.1
<b>9</b>	3.9	5.1	3.3	2.6	1.4	1.7	1.3	2.0
<b>10</b>	3.8	2.6	2.9	1.8	1.6	3.1	2.4	2.4
<b>11</b>	3.1	2.9	2.6	1.6	1.7	2.3	2.1	5.0
<b>12</b>	3.5	3.1	2.4	1.4	1.9	2.4	2.4	1.6
<b>13</b>	3.2	2.8	2.6	1.4	0.6	2.0	1.1	1.0
<b>14</b>	3.3	3.6	3.1	2.8	1.2	1.6	2.2	1.7
<b>15</b>	4.2	5.6	3.9	4.1	3.9	3.2	4.6	4.0
<b>16</b>	3.1	3.0	2.4	3.7	0.8	1.4	1.7	2.7
<b>17</b>	3.0	2.0	2.2	1.7	1.8	1.3	3.1	2.6
<b>18</b>	3.4	3.2	2.1	2.3	4.1	1.8	1.4	2.3
<b>19</b>	3.8	3.4	3.7	2.8	1.3	3.0	3.9	3.7
<b>20</b>	3.6	2.2	2.5	2.6	1.7	2.2	2.2	1.6
<b>21</b>	3.5	3.4	3.1	3.9	4.8	1.5	1.7	3.4
<b>22</b>	4.0	4.7	3.3	4.6	4.7	5.6	5.5	4.8
<b>23</b>	3.1	3.5	1.9	2.8	3.7	1.4	1.7	1.8
<b>24</b>	3.2	5.3	2.8	2.1	3.6	1.8	4.4	3.7
<b># out of 24</b>	24/24	24/24	24/24	17/24	14/24	17/24	12/24	15/24
<b>% Positive</b>	100%	100%	100%	71%	58%	71%	50%	63%

100 **Table S6.** Correlation between the levels of IgA antibodies against different mammalian and  
 101 plant milks shown as ELISA index.

<b>IgA</b>	<b>Cow</b>	<b>Goat</b>	<b>Sheep</b>	<b>Camel</b>	<b>Human</b>	<b>Soy</b>	<b>Almond</b>	<b>Coconut</b>
<b>1</b>	3.4	1.2	1.3	1.5	*	2.2	1.8	0.8
<b>2</b>	3.5	1.5	1.2	2.1	*	0.8	1.1	0.6
<b>3</b>	3.0	1.7	1.6	1.6	*	0.9	1.5	0.9
<b>4</b>	3.1	1.8	1.8	1.2	*	1.9	1.6	0.7
<b>5</b>	4.2	2.3	2.0	5.6	*	1.6	1.7	0.8
<b>6</b>	6.1	6.5	5.1	2.0	*	1.9	2.8	2.3
<b>7</b>	3.4	1.5	1.8	1.1	*	2.3	2.4	0.9
<b>8</b>	3.9	1.3	1.7	2.6	*	1.9	1.7	3.6
<b>9</b>	4.3	3.4	1.6	1.6	*	2.4	2.3	1.4
<b>10</b>	4.5	1.8	1.5	1.4	*	2.0	2.1	2.0
<b>11</b>	4.2	4.3	1.7	1.7	*	1.4	1.8	1.4
<b>12</b>	4.3	2.6	1.8	3.3	*	3.3	1.2	0.9
<b>13</b>	3.6	1.0	1.2	1.1	*	1.0	1.0	1.1
<b>14</b>	3.1	2.6	1.1	2.5	*	1.2	1.2	0.8
<b>15</b>	4.1	1.1	1.0	2.0	*	1.7	1.5	1.2
<b>16</b>	5.1	2.6	2.2	2.2	*	2.4	2.3	1.7
<b>17</b>	6.9	7.2	7.3	0.6	*	0.6	0.7	1.1
<b>18</b>	4.2	1.3	1.7	1.4	*	1.3	1.2	1.8
<b>19</b>	7.0	5.5	4.9	2.9	*	5.3	6.0	5.4
<b>20</b>	3.7	2.9	2.3	5.1	*	1.9	2.2	1.7
<b>21</b>	3.5	5.1	2.1	2.1	*	1.2	1.6	1.4
<b>22</b>	3.1	4.8	2.7	3.9	*	1.9	1.7	1.1
<b>23</b>	2.9	1.6	1.5	2.0	*	1.2	1.0	1.2
<b>24</b>	3.0	2.5	1.4	2.0	*	2.4	2.5	1.3
<b># out of 24</b>	24/24	16/24	14/24	15/24	*	14/24	14/24	7/24
<b>% Positive</b>	100%	67%	58%	63%	*	58%	58%	29%

102 \* When human serum was added to the wells coated with human milk antigen, the antibodies  
 103 reacted so strongly with the human IgA that no accurate measurement was possible.  
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107 **Table S7. IgA Risk Analysis**

<b>Risk of Reactivity to Other Milk Proteins IgA if Cow's Milk IgA is Reactive</b>		
<b>Milk Protein Source</b>	<b>Risk Ratio</b>	<b>P-value</b>
Goat Milk IgA	9 (6-13)	< 0.0001
Sheep Milk IgA	7 (4-12)	< 0.0001
Camel Milk IgA	3 (2-6)	0.002
Almond Milk IgA	no statistically significant risk	
Human Milk IgA	no statistically significant risk	
Soy Milk IgA	no statistically significant risk	
Coconut Milk IgA	no statistically significant risk	

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109 **Table S8. IgE Risk Analysis**

<b>Risk of Reactivity to Other Milk Proteins if Cow's Milk is Reactive</b>		
<b>Milk Protein Source</b>	<b>Risk Ratio</b>	<b>P-value</b>
Goat Milk IgE	6 (1.5-26)	< 0.006
Sheep Milk IgE	9 (3-30)	< 0.0001
Camel Milk IgE	no statistically significant risk	
Almond Milk IgE	no statistically significant risk	
Human Milk IgE	no statistically significant risk	
Soy Milk IgE	no statistically significant risk	
Coconut Milk IgE	no statistically significant risk	

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111 **Table S9. IgG Risk Analysis**

<b>Risk of Reactivity to Other Milk Proteins IgG if Cow's Milk IgG is Reactive</b>		
<b>Milk Protein Source</b>	<b>Risk Ratio</b>	<b>P-value</b>
Goat Milk IgG	9 (6-13)	< 0.0001
Sheep Milk IgG	7 (4-12)	< 0.0001
Camel Milk IgG	3 (2-6)	0.002
Almond Milk IgG	no statistically significant risk	
Human Milk IgG	no statistically significant risk	
Soy Milk IgG	no statistically significant risk	
Coconut Milk IgG	no statistically significant risk	

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113 **Table S10. IgA Correlations**

<b>Correlation of Cow's Milk IgA Antibodies with Other Milk Proteins IgA</b>		
<b>Milk Protein Source</b>	<b>Coefficient</b>	<b>P-value</b>
Goat Milk IgA	0.8	<0.0001
Sheep Milk IgA	0.6	<0.0001
Almond Milk IgA	0.3	<0.0001
Camel Milk IgA	0.4	<0.0001
Human Milk IgA	0.05	0.2457
Soy Milk IgA	0.3	<0.0001
Coconut Milk IgA	0.2	<0.0001

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115 **Table S11. IgE Correlations**

<b>Correlation of Cow's Milk IgE Antibodies with Other Milk Proteins</b>		
<b>Milk Protein Source</b>	<b>Coefficient</b>	<b>P-value</b>
Goat Milk IgE	0.7	<0.0001
Sheep Milk IgE	0.7	<0.0001
Almond Milk IgE	0.4	<0.0001
Camel Milk IgE	0.4	<0.0001
Human Milk IgE	0.4	<0.0001
Soy Milk IgE	0.5	<0.0001
Coconut Milk IgE	0.4	<0.0001

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117 **Table S12. IgG Correlations**

<b>Correlation of Cow's Milk IgG Antibodies with Other Milk Proteins IgG</b>		
<b>Milk Protein Source</b>	<b>Coefficient</b>	<b>P-value</b>
Goat Milk IgG	0.8	<0.0001
Sheep Milk IgG	0.7	<0.0001
Almond Milk IgG	0.3	<0.0001
Camel Milk IgG	0.6	<0.0001
Human Milk IgG	0.5	<0.0001
Soy Milk IgG	0.4	<0.0001
Coconut Milk IgG	0.4	<0.0001

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