Milk and dairy intake and the metabolic syndrome
Milk and Dairy Intake and the Metabolic Syndrome

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Abstract
The overall aim of this master thesis was to get an overview on how milk and dairy consumption affect development of the metabolic syndrome, and from this review to formulate a milk product with potentially beneficial effects.

A cluster of metabolic abnormalities such as insulin resistance and type 2 diabetes, hypertension, obesity and dyslipidaemia are known as the metabolic syndrome. Epidemiological studies performed to investigate the relation between milk and dairy intake and the metabolic syndrome, suggests that low-fat milk and dairy intake have a positive effect in the prevention of the disease. Many dairy components might contribute to this effect.

There are promising effects seen by whey amino acids on the glucose and insulin control, but the long-term effects are warranted. Low-fat milk and dairy as part of a diet rich in fruits and vegetables have the most blood pressure reducing effect. This beneficial effect is in part believed to be due to the calcium content of milk and dairy products. In addition, it is also hypothesised that calcium plays an important role in weight management. However, the evidence up to date is contradictorily.

Weight control, on the other hand, can be improved by affecting satiety. Acute intervention studies show that whey, in particular, alfa-lactalbumin, is more satiating than other proteins, resulting in a lower energy intake in a subsequent meal.

It is of interest to the dairy industry to provide milk and dairy consumers with milk products that have beneficial effects on wellness and health. Therefore, based on the literature reviewed on milk and dairy intake and the metabolic syndrome, a milk product with beneficial effects on weight was formulated and developed.
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**Sammanfattning**

Syftet med detta examensarbete var att sammanfatta kunskapsläget kring hur intag av mjölk påverkar det metabola syndromets olika delkomponenter samt att utifrån litteraturstudien identifiera en intressant mjölkkomponent/fraktion och ge förslag på en möjlig produkt.

Metabola syndromet är ett samlingsnamn för olika kardiovaskulära riskfaktorer där folkhälsosjukdomar som insulin resistens och diabetes typ 2, hypertension, övervikt och dyslipidemi ingår.

Epidemiologiska studier som undersökt sambandet mellan intag av mjölk och mjölkprodukter och det metabola syndromet visar att mjölk och mjölkprodukter med ett lågffett innehåll kan verka positivt i preventionen av sjukdomen. Olika mjölkkomponenter kan bidra med denna effekt. Grenade aminosyror från vassle har visat sig ha en positiv effekt på kort sikt på glukos och insulin kontroll, men långtidsresultaten är ännu inte klarlagda.

Mjölk och mjölkprodukter med ett lågffett innehåll som en del av en hälsoaktiv kost rik, på frukt och grönsaker, har visats sig ha den mest blodtryckssänkande effekten. Denna positiva effekt på blodtrycket tror man till viss del beror på kalciuminnehållet i mjölk och mjölkprodukter. Dessutom verkar kalcium kunna spela en viktig roll vid viktreglering.

Evidensen som styrker denna tes är dock motsägelserfull.


Det är viktigt för mjölk och mejeriindustrin att förse mjölkdrickande konsumenter med mjölkprodukter som påverkar hälsan och människans välmående positivt.

I detta syfte och med den veteskapliga litteraturen som bas, har en mjölkprodukt som har effekt på mättnad formulerats och utvecklats.
Preface

This master thesis was performed as part of the Master Degree program in Nutrition and Food Science at the Linneaus University in Kalmar.
All practical work was conducted in the laboratories and pilot plant of Arla Foods Global Fresh Dairy Products Innovation in Stockholm, Sweden.

First, I would like to thank my supervisor Karin Arkbåge at Arla Foods Global Fresh Dairy Products Innovation in Stockholm for her outstanding support and tremendous help throughout this work. I would also like to thank Anna Asplund for her feedback and shaping of this report. At last, special thanks to Ulla Svensson, Janet Håkansson, Eva Öhman and the Bioscience group for letting me take part of your inspiring activities and interesting meetings. It has been a real adventure from the beginning to the end performing this work, and I have learnt so much!

Gabriela García Bravo
Stockholm May 28, 2010
1. Aim

The overall aim of this master thesis was to get an overview on how milk and dairy consumption affect development of the metabolic syndrome, and from this review to formulate a milk product with potentially beneficial effects.

The work was divided into three parts. Step one, the major part of the thesis, was to review and summarise existing literature investigating the effect of milk and dairy consumption on development of the metabolic syndrome. This was done with special emphasis on three of the components of the metabolic syndrome: insulin resistance and diabetes type 2, hypertension and central abdominal obesity.

The second step, based on the literature review, was to identify a milk component or milk fraction with potentially beneficial effect on any of the three components of the metabolic syndrome.

The last step involved formulation of a milk product containing the milk component/fraction identified in step two. The practical work was performed in laboratory scale and also in a pilot plant scale.

2. Introduction

2.1 The definition of the metabolic syndrome

The metabolic syndrome and its underlying risk factors have developed to a fast and growing problem in the field of public health. The syndrome is often described as a cluster of risk factors that together contribute to a dangerous health state. The pathogenesis of the syndrome is complex and so far incompletely understood; but the interaction of obesity, sedentary lifestyle, and dietary and genetic factors are known to contribute to its development. Persons with the metabolic syndrome have an increased risk of death from all causes as well as cardiovascular disease. (Hu et al. 2004)

Among the adult population in many Western societies the prevalence of the metabolic syndrome exceeds to nearly 20% and it is expected to increase in the future (Van Meijl et al. 2008).

The definition of the metabolic syndrome is not agreed internationally but several attempts to define the syndrome have been done. A clear definition is needed both as a research tool, and also as a tool to facilitate diagnosis of the metabolic syndrome by clinicians. There exist definitions proposed by various international organs differing from each other in small aspects but all of them agreeing on following main components of the metabolic syndrome:

- Raised fasting glucose, glucose intolerance or diabetes type 2
- Hypertension
- Central abdominal fat
- Dyslipidaemia (raised triglycerides levels and low HDL-cholesterol levels)

(Nilsson et al. 2006).
Table 1. The metabolic syndrome definition by various organizations.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definition</strong></td>
<td>Glucose intolerance, impaired glucose tolerance (IGT) or diabetes and/or insulin resistance, plus any two or more of the following components. (<em>Focus on diabetes</em>)</td>
<td>Central abdominal fat plus any two of the following components (<em>Focus on abdominal fat</em>)</td>
<td>Any three or more of the following components (<em>Focus on lipid values</em>)</td>
<td>Insulin resistance plus any two or more of the following components (<em>Focus on insulin resistance</em>)</td>
<td>To identify subjects at risk they must have at least one of the following criterias</td>
</tr>
<tr>
<td><strong>Glucose metabolism</strong></td>
<td>Fasting plasma glucose ≥ 6.1 mmol/l. Reduced glucose tolerance (OGTT)</td>
<td>Elevated fasting glucose ≥ 5.6 mmol/l and/or diagnosed diabetes type 2</td>
<td>Fasting glucose ≥ 6.1 mmol/l</td>
<td>Fasting glucose (nondiabetic) ≥ 6.1 mmol/l</td>
<td>Fasting plasma glucose ≥ 6.1-6.9 mmol/l</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td>≥140/90 mmHg</td>
<td>≥130/85 mmHg or treatment of previously diagnosed hypertension</td>
<td>≥130/85 mmHg or treatment</td>
<td>≥140/90 mmHg or treatment</td>
<td>&gt; 130/85 mmHg</td>
</tr>
<tr>
<td><strong>Dys-lipidaemia</strong></td>
<td>Triglycerides; ≥ 1.7 mmol/l and/or low HDL—cholesterol ≤0.9 mmol/l for men; &lt; 1.0 mmol/l for women</td>
<td>Triglycerides; ≥ 1.7 mmol/l. Reduced HDL—cholesterol &lt; 1.03 mmol/l for men and &lt; 1.29 mmol/l for women</td>
<td>Triglycerides; ≥ 1.7 mmol/l</td>
<td>Triglycerides; ≥ 2.0 mmol/l or HDL—cholesterol &lt; 1.0 mmol/l for treatment of dyslipidaemia</td>
<td>Triglycerides &gt; 1.7 mmol/l HDL—cholesterol men; &lt; 1.0 mmol/l; women &lt; 1.3 mmol/l</td>
</tr>
<tr>
<td><strong>Central abdominal fat</strong></td>
<td>Waist to hip ratio for men &gt; 0.90; female &gt; 0.85 and/or BMI &gt; 30 kg/m²</td>
<td>Central abdominal fat ≥ 94 cm for European men and ≥ 80 cm for European women, specific for different ethnic groups</td>
<td>Mid waist circumference ≥ 102 cm for men and 88 cm for women</td>
<td>Waist circumference ≥ 94 cm in men and ≥ 80 cm in women</td>
<td></td>
</tr>
<tr>
<td><strong>Vascular injury</strong></td>
<td>Microalbuminuria; urinary albumin excretion rate ≥ 20 μg/min or albumin:creatinine ratio ≥ 30 mg g⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* WHO: World Health Organisation  
* IDF: International Diabetes Federation  
* NCEP: National Cholesterol Education Programme: Adult treatment panel III  
* EGIR: The European Group for the study of Insulin Resistance  
* AACE: The American Association of Clinical Endocrinology

The WHO and EGIR classifications require the measurement of insulin resistance, which is determined by an oral glucose tolerance test and hyperinsulinaemic-euglycaemic clamp. As this method is lab intensive, it is primarily used in research environment. In contrast, the NCEP definition, with focus on cardiovascular disease risk, was developed to be applicable in the outpatient clinic and therefore have remained a backbone for subsequent classifications such as the IDF diagnostic criterion. The IDF definition aims to identify people at high risk of cardiovascular disease, but also diabetes. This definition allows also comparative long-term studies. In 2003 the American Association of Clinical Endocrinology modified the NCEP definition. No specific numbers of factors qualified for diagnosis, which is left to the clinical judgement. (Bruce et al. 2009, Alberti et al. 2005, Grundy et al. 2005)
2.2 Milk and its components

The role of milk in nature is to provide the mammalian newborn with nutrition and immunological protection. Milk is a complex matrix composed of a mixture of carbohydrates, proteins, lipids, minerals and vitamins. The main components in bovine milk are viewed in table 2.

As an oil-in-water emulsion, milk has an average percentage of 87% water and 4,1% fat. (Damodaran et al. 2007)

<table>
<thead>
<tr>
<th>Component</th>
<th>Average percentages of each component</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>86,6</td>
</tr>
<tr>
<td>Fat</td>
<td>4,1</td>
</tr>
<tr>
<td>Protein</td>
<td>3,6</td>
</tr>
<tr>
<td>Lactose</td>
<td>5,0</td>
</tr>
<tr>
<td>Ash</td>
<td>0,7</td>
</tr>
</tbody>
</table>

(Damodaran et al. 2007)

Figure 1. Milk viewed at different magnifications. The picture indicates the relative size of structural elements. Observe the size of the casein molecules that are much smaller that the fat molecule.

A) Uniform liquid. However, the liquid is turbid and thus cannot be homogenous.

B) Spherical droplets, consisting of fat. These globules float in a liquid (plasma), which is turbid.

C) The plasma contains proteinaceous particles, which are casein micelles. The remaining liquid (serum) is still opalescent, so it must contain other particles. The fat globules have a thin outer layer (membrane) of different constitution.

(Adapted and modified from Walstra et al. 1999)

The role of milk in the traditional diet varies greatly in different regions of the world. Milk drinking habits has not been common in the tropical countries, whereas in the Northern regions of the world, Europe (especially Scandinavia) and North America, milk is included as a natural part of the diet. Also within regions in Europe, the milk product consumption pattern varies a lot. To exemplify, the consumption of fluid milk in countries like Finland, Norway
and Sweden is high compared to France and Italy, where cheeses consumption dominate. (International Dairy Federation, Bulletin 423/2007)

In the past there was only whole milk (milk without fat standardization), but the dairy industries commenced to develop milk with different fat content as the consumer requested it. In 1969 Arla Foods launched low-fat milk with 0,5% fat content which quickly became a popular product. Swedish milk range consists of milk with different fat content from whole milk with 4,6% fat to very low-fat milk with only 0,1% fat. (www.arlafoods.se)

2.2.1 The nutritional value of milk

The nutritional value of milk is high, providing 18 of the 22 essential nutrients needed daily recommended by the Nordic Nutrition Recommendations (NNR 2004). As shown in figure 2 by drinking 0,5 L of low-fat milk or corresponding amount of dairy products per day, will provide the body with 75–80% of phosphor and calcium, and more than 100% of vitamin B12. (NNR 2004, Mjölkfrämjandet 2010).

Figure 2. Nutrient content of 0,5 L of milk 1,5% fat content. The data as given in percentage of recommended daily intake, NNR 2004.

(Mjölkfrämjandet 2010)

2.2.2 Milk carbohydrates

Milk has a relatively low carbohydrate content (5,0%) and lactose is the major carbohydrate in milk. The sweetness of lactose is about one-fifth of that of sucrose. Some individuals have the inability to metabolize this molecule, a condition called lactose intolerance. Lactose intolerance means that the enzyme β-galactosidase (lactase) is lacking and therefore the reaction where the lactose molecule is hydrolysed is unable to occur. The lack of lactase gives
symptoms such as diarrhea, bloating, and abdominal cramps. To be able to provide lactose intolerant individuals with milk, lactose is hydrolyzed in milk during process, resulting in lactose reduced and/or lactose free milk. (There are also lactase tablets available in the market to take before meal with lactose containing milk products.) (Damodaran et al. 2007)

2.2.3 Milk proteins
Milk proteins consist of whey proteins (20%) and caseins (80%). The major whey proteins are β-lactoglobulins and α-lactalbunins. For an overview of the concentration of proteins in milk see table 3. The group of casein consist of αs1-caseins, αs2-caseins, β-caseins, and κ-caseins. Besides these two types of proteins in milk there are other protein components that in fact are large polypeptides. These polypeptides are a result from a posttranslational proteolysis of whey and casein proteins made by the milk proteinase plasmin. Therefore γ-caseins and protease-peptones, both present in whey, are a result of the proteolysis of β-casein. Casein proteins exist with calcium phosphate in a hydrated spherical complex named the casein micelle. The casein micelle consists of 92 % proteins in the ratio 3:1:3:1 of αs1: αs2: β-κ-caseins. The micelle contains 8 % calcium phosphate, magnesium ions and citrate, all together known as milk salts. (Damodaran et al. 2007)

Table 3. Concentrations of the major proteins in bovine milk.

<table>
<thead>
<tr>
<th>Protein component</th>
<th>Amount (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>3,37</td>
</tr>
<tr>
<td>Whey</td>
<td>0,62</td>
</tr>
<tr>
<td>β-lactalbunin A</td>
<td>0,22</td>
</tr>
<tr>
<td>β-lactalbunin B</td>
<td>0,13</td>
</tr>
<tr>
<td>α-lactalbunin</td>
<td>0,10</td>
</tr>
<tr>
<td>Bovine serum albumine (mg/100g)</td>
<td>25</td>
</tr>
<tr>
<td>Lactoferrin (mg/100g)</td>
<td>9</td>
</tr>
</tbody>
</table>

(Lindmark-Månsson 1996)

2.2.4 Milk lipids
Milk fat is a complex fat consisting of up to 400 different fatty acids. Therefore it is complicated to find adequate substitutes to milk fat in nonfat or low-fat milk products. Bovine milk fat is derived those from acetic acid produced by microorganisms present in the rumen of the cow, and in the udder from hydroxybutyrate. Triacylglycerol (triglycerides), is the major lipid type in bovine milk, comprising nearly 96 % of the total fat. The lipid molecules associate to form spherical globules. The plasma membrane of the fat globules consists of a coat of cell membrane proteins including enzymes, nearly 80 % of the cholesterol and 70 % of the phospholipids in milk (table 4).

Table 4. Lipids composition of bovine milk. The given data is representative for fresh milk

<table>
<thead>
<tr>
<th>Lipid component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat content (g/100g)</td>
<td>4,34</td>
</tr>
<tr>
<td>Free fatty acids mekv/l</td>
<td>0,60</td>
</tr>
<tr>
<td>Cholesterol mg/100g</td>
<td>13,9</td>
</tr>
<tr>
<td>Phospholipids mg/100g</td>
<td>21,3</td>
</tr>
</tbody>
</table>

(Lindmark-Månsson 1996)
2.2.5 Vitamins in milk

Milk contains many vitamins both fat and water soluble. The water soluble can act as cofactors or precursors of cofactors. This is the case for the water-soluble vitamins thiamin (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), cobalamin (B12) and folates. (Damodaran (2007), Mjölkfrämjandet (2010). The quantity of the different vitamins in 100g of milk (1.5%) is viewed in table 5.

The fat soluble vitamins in milk are vitamin A and D. When making fat reduced milk, vitamin A is added to compensate the loss during the process. Milk is enriched with vitamin D (Damodaran 2007). Milk and milk products stands for 11 % of vitamin A and 15 % of vitamin D intake in Swedish adults (Mjölkfrämjandet 2010)

Table 5. The vitamin content in Swedish “mellanmjölk” 1,5 fat %/100g and the contribution of vitamin intake from milk in the Swedish diet.

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Quantity/100g</th>
<th>Proportion of the intake covered by milk intake in the Swedish diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>39,6 µg</td>
<td>11 %</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>0,03 µg</td>
<td>15-20 %</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>101 µg</td>
<td>-</td>
</tr>
<tr>
<td>Thiamin, B1</td>
<td>40 µg</td>
<td>15 %</td>
</tr>
<tr>
<td>Riboflavin, B2</td>
<td>141 µg</td>
<td>35-40 %</td>
</tr>
<tr>
<td>Niacin, B3</td>
<td>64 µg</td>
<td>14 %</td>
</tr>
<tr>
<td>Pyridoxin, B6</td>
<td>42 µg</td>
<td>10 %</td>
</tr>
<tr>
<td>Cobalamin, B12</td>
<td>0,41 µg</td>
<td>23 %</td>
</tr>
<tr>
<td>Folates, B12</td>
<td>5,6 µg</td>
<td>15 %</td>
</tr>
</tbody>
</table>

(Lindmark-Månsson 1996)

2.2.6 Minerals in milk

Not only is milk rich in vitamins it is also a rich source of minerals. Milk and milk products cover the main part of the calcium requirements in adults. These products are the most important food source for this mineral. Nearly two third of the calcium in milk is bound to the casein micelles either as calcium phosphate or calcium ions in a colloidal form. Table 6 illustrates the mineral content of Swedish “mellanmjölk” 1,5 % fat /100g.

Table 6. The mineral content in Swedish “mellanmjölk” 1,5 % /100g. The right column of the table shows in percentage the proportion of the intake that’s covered by milk.

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Quantity/100g</th>
<th>Proportion of the intake covered by milk intake in the Swedish diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>114 mg</td>
<td>63 %</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>90 mg</td>
<td>35 %</td>
</tr>
<tr>
<td>Iodine</td>
<td>14 µg</td>
<td>-</td>
</tr>
<tr>
<td>Potassium</td>
<td>160 mg</td>
<td>16 %</td>
</tr>
<tr>
<td>Magnesium</td>
<td>12 mg</td>
<td>16 %</td>
</tr>
<tr>
<td>Selenium</td>
<td>1,8 µg</td>
<td>17 %</td>
</tr>
<tr>
<td>Zinc</td>
<td>0,44 mg</td>
<td>22 %</td>
</tr>
</tbody>
</table>

(Mjölkfrämjandets 2010, Lindmark-Månsson 1996)
3. Method to select the literature

A PUBMED search was conducted for studies published in English over the last 13 years (January 1997 to January 2010) on the relationship between milk and dairy intake and the components of the metabolic syndrome. The key words, milk/milk intake/milk consumption, dairy/dairy intake/dairy consumption, were combined with each of the component of the metabolic syndrome; insulin resistance and/or diabetes type 2, hypertension, and overweight/obesity. The main focus was observational studies intervention studies and animal studies for each of the components of the metabolic syndrome. How the fourth component of the metabolic syndrome, dyslipidaemia is affected by milk and dairy intake is not included in this work. There is a large amount of both observational and intervention studies performed on the relationship between specific fatty acids and blood lipids. However, there are few studies on the relationship between milk and dairy as a total nutrient and its relation to blood lipids.

4. The metabolic syndrome; a review of the literature

4.1 Effects of milk and dairy intake on the metabolic syndrome in total

A cluster of metabolic disorders that are risk factors for cardiovascular disease and diabetes type 2 characterizes the Insulin Resistance Syndrome (IRS), otherwise known as the syndrome X or the metabolic syndrome. Around the world, the prevalence of diabetes type 2 is increasing and it is closely connected to the rising rates of obesity. Heredity influences the development of the metabolic syndrome but lifestyle choices and health behaviours, which are modifiable, also play a major role in the development and progression of the metabolic syndrome. Key recommendations for preventing and managing the metabolic syndrome include weight loss, increased physical activity and dietary changes. The recommended dietary changes include a reduction in calories while developing healthy eating habits that incorporate a diet low in saturated fat, trans fat and cholesterol, and an increase in consumption of low fat dairy products, fruits and vegetables, and/or whole grains. (Tremley 2009, Pfeuffer et al. 2006, Van Meijl et al. 2008) Several epidemiological studies have investigated the relationship between milk and dairy consumption and the metabolic syndrome (Pereira et al. 2002, Snijder et al. 2007, Elwood et al. 2007, Beydoun et al. 2008, Azadbakht et al. 2005, Liu et al. 2005). A majority of these studies show an inverse relationship between dairy intake and the components of the metabolic syndrome. One study shows however that avoiding milk could protect from developing insulin resistance and the metabolic syndrome (Lawlor et al. 2005).

4.2 Effects of milk and dairy intake on insulin resistance and diabetes type 2

Diabetes type 2 affects nearly 16 million people in the U.S and 135 million people worldwide. This number of affected individuals is expected to increase and reach an estimated 300 million people globally by 2025. The disease, because of its complications such as cardiovascular disease, amputation, blindness and renal failure, imposes enormous medical and economical burdens. As a result primary prevention of diabetes type 2 has become a public health imperative. (Choi et al. 2005)

Whey proteins appear to modify both insulinemic and glycemic responses to carbohydrate-rich food in both type 2 diabetic and healthy subjects. Intervention studies investigating this
effect show that branched-chain amino acids (leucine, isoleucine, valine, lysine and treonine) are the major determinants of insulinemia as well as lowered glycemia caused by whey. However, performed studies are acute, short-term studies, and well-performed randomized controlled trials need to conclude the long-term effects of whey proteins. (Luhovny et al. 2007, Liljeborg et al. 2001, Nilsson et al. 2004, Petersen et al. 2009, Nilsson et al. 2007, Frid et al. 2005).

There is mechanistic support that vitamin D may influence insulin secretion and insulin sensitivity and subsequent type 2 diabetes. In general, cross-sectional and prospective studies support the role of vitamin D in the prevention of type 2 diabetes. Despite the inherent limitations of cross-sectional and prospective study designs, these types of study designs are useful for preliminary research to suggest which specific populations may respond to vitamin D interventions. However, large, well-controlled, randomized studies are required to clarify important unknowns and define the relationship between vitamin D status and glucose homeostasis. (Alvarez et al. 2010)

The relationship between milk and dairy intake and the risk of suffering from insulin resistance or diabetes type 2 has been investigated in several epidemiological studies (Choi et al. 2005, Liu et al. 2006, Hirschler et al. 2009). A majority of studies show a negative association between milk and dairy consumption and the incidence of insulin resistance and diabetes type 2. However, one study shows a positive association (Papakonstantinou et al. 2005).

4.3 Effect of milk and dairy intake on blood pressure

High blood pressure is a major risk factor for stroke, heart disease, congestive heart failure and kidney disease.

In a recent review by Kris-Etherton et al. (2009) dairy nutrients, most notably calcium, potassium, and magnesium, are highlighted to possess blood pressure lowering effect.

A growing base of evidence shows that low-fat dairy product consumption is involved in the regulation of blood pressure. Consequently, inclusion of dairy products in a heart healthy diet is an important focal point to attain blood pressure benefits. (Kris-Etherton et al. 2009)

A review by Zemel (2001) suggests that the mechanism for how calcium affects blood pressure is explained by the role of calcitrophic hormones. Increased intracellular calcium increases vascular smooth muscle tone, peripheral vascular resistance, and blood pressure, while an increase of dietary calcium has the opposite effect. The effect of 1,25-dihydroxyvitamin D stimulation on calcium influx is rapid, and it is mediated by the membrane vitamin D receptor. 1,25-dihydroxyvitamin D also promotes contraction and increases peripheral vascular resistance. Therefore, a low dietary calcium intake increases intracellular calcium concentrations, which increases 1,25-dihydroxyvitamin D₃ and parathyroid hormone, causing calcium influx into vascular smooth muscle cells, resulting in a greater vascular resistance. In contrary, high calcium diet are thought to suppress 1,25-dihydroxyvitamin D and would be expected to reduce vascular smooth muscle cell intracellular calcium, peripheral vascular resistance and blood pressure. (Zemel 2001)

Furthermore, new research indicates that dairy peptides (lactotripeptides) may act as angiotensin converting enzyme (ACE) inhibitors, thereby inhibiting the renin angiotensin system with consequent vasodilation. (Kris-Etherton et al. 2009)
Milk peptides are formed when milk proteins are broken down by digestive enzymes or by the proteinases formed by lactobacilli during fermentation of milk. It has been suggested that peptides with hydrophobic amino acids at the C-terminal position could be the most likely ACE-inhibitors. Isoleucine-proline-proline (IPP) and valine-proline-proline (VPP), derived from β-casein and κ-casein by enzymes from *Lactobacillus helveticus*, have an ACE inhibitory effect (Hartmann *et al.* 2007, Jauhiainen *et al.* 2007).

ACE is an important regulator of blood pressure. The juxtaglomerular apparatus in the nephron secretes renin in response to a decreased circulating blood volume and also by sympathetic nervous stimulation. This protease cleaves angiotensin I which is then converted to angiotensin II by ACE. After binding to its membrane, angiotensin II activates protein kinase C and the production of inositol triphosphate. This results in the mobilization of stored Ca^{2+} and free Mg^{2+} into the cytoplasm. Therefore elevated levels of angiotensin II can lead to elevated levels of intracellular Ca^{2+} and lowered intracellular Mg^{2+} concentrations, resulting in vasoconstriction. (Kris-Etherton *et al.* 2009)

In a review by Boelsma *et al.* (2009), summarizing the antihypertensive effects of lactotripeptides, conclude that lactotripeptides (IPP and VPP) needs to be consumed continuously for at least 1-2 weeks before blood pressure effects become apparent. Within the first two weeks the largest effect is seen. This was followed by a gradual blood pressure lowering effect until a maximum effect after about 8-12 weeks of treatment were achived. A maximum blood pressure reduction of approximate 13 mmHg in the systolic blood pressure and 8 mmHg in the diastolic blood were obtained after active treatment compared with placebo. Effective dosages of lactotripeptides range from 3,07 to 52,5 mg/day. Furthermore, given the fact that lactotripeptides are safe and exert relevant and stable blood pressure lowering effects within a relatively short period of time, they are good candidates to be included in healthy lifestyle changes to prevent or reduce high blood pressure. (Boelsma *et al.* 2009)

Several epidemiological studies investigate the relationship between milk and dairy consumption and hypertension. Results from a majority of these studies show an inverse association between dairy consumption and the risk of hypertension. (Pfeuffer *et al.* 2006, Engberink *et al.* 2009, Djourssé *et al.* 2006, Alonso *et al.* 2009, Jorde *et al.* 2000)

However, a few studies show a neutral effect of milk and dairy intake on blood pressure (Engberink *et al.* 2008, Van der Zander *et al.* 2008)

### 4.4 Effects of milk and dairy intake on central abdominal fat

Overweight is one of the main criteria included in the definition of the metabolic syndrome. Several studies have reported that the incidence of metabolic syndrome increases with the severity of obesity. Type 2 diabetes is five to six times more common in obese people (BMI >30kg/m²) than in those of normal weight. This increasing BMI is also correlated with other components of the metabolic syndrome such as hypertension, increased levels of total cholesterol, low-density lipoprotein (LDL)-cholesterol and triacylglycerol and decreased levels of HDL-cholesterol. The most common contributor to the rise in metabolic syndrome is excessive body fat accumulation, more specifically ectopic fat. This results in a pathophysiological condition named adiposopathy, which is defined as adipose tissue that is promoted by a sedentary lifestyle and positive energy balance in genetically and environmentally susceptible patients. It is believed that adiposopathy is a combination of adipocyte hypertrophy, adipose tissue growth, ectopic fat distribution and visceral adipose
tissue accumulation, all of which may contribute to the development of the metabolic syndrome. An increased visceral fat accumulation gives rise to elevated production of proinflammatory adipocytokines which contributes to the development of insulin resistance, type 2 diabetes and increased risk of cardiovascular disease. (Bruce et al. 2009)

Whey proteins have potential as functional food components in contributing to regulation of body weight by providing satiety signals that affect both short-term and long-term food intake regulation. At present, the role of individual whey proteins and peptides in contributing to food intake regulation has not yet been fully defined. Whey proteins affects satiation and satiety by the actions of whey proteins per se, or by amino-acids released after digestion, or by a combined action of whey proteins and/or peptides and/or amino acids with other milk components. Therefore, whey proteins have potential as physiologically interesting food component for persons with obesity and the metabolic syndrome. It remains unclear, though, if the favorable effects of whey on food intake, subjective satiety and intake regulatory mechanisms in humans are obtained from usual serving sizes of dairy products. (Luhovyy et al. 2007)

The effect of dietary calcium on obesity has been discussed in several reviews. These reviews are suggesting that a high calcium intake is associated with a lower body weight and a lower fat mass. Furthermore, it has been proposed that calcium from dairy sources exerts larger effects on fat loss than calcium from non-dairy sources. There are theories that there can also be other bioactive compounds in dairy products that may act synergistically with calcium and affect body weight and fat mass. (Van Meijl et al. 2008, Major et al. 2008, Astrup et al. 2008, Van Loan et al. 2009, Zemel et al. 2005)

Although, several observational and intervention studies show a positive effect of dairy, there are also a few studies where no anti-obesity effect of calcium has been shown. A review article by Lanou et al. (2008), that summaries 49 randomized trials assessing the effect of dairy products or calcium supplementation on body weight, conclude that 41 showed no effect, two demonstrated weight gain, one showed a lower rate of gain, and five showed weight loss. As a result authors conclude that there is no evidence that supports the hypothesis that dairy or calcium consumption alone or in conjunction with caloric restriction, results in weight or fat loss in the short or long term. (Lanou et al. 2008)
5. Discussion of the literature study

In general, for studies of observational design (cross-sectional-, cohort-, and case control studies), residual confounding factors cannot be ruled out. Therefore the conclusion that an increase/decrease of milk and dairy consumption is positively or negatively associated with the components of the metabolic syndrome cannot be drawn in a causal manner.

To assess dietary intake, study participants in observational studies most often self-report their intake of milk and dairy by semi-quantitative questionnaires. This might lead to some misclassification of milk and dairy consumption. This error in measurement of dairy intake are likely to bias towards the null hypothesis, thus towards no association between dairy intake and the components of the metabolic syndrome, which can lead to an underestimation of the true magnitude of the association.

The studies of observational design are performed to search for a relationship between a specific factor that affect health and illness in a studied population. If a relationship is found in an observational study, for example between milk consumption and the metabolic syndrome, this relationship must be tested in an intervention study to be able to conclude that a cause and effect relationship exists.

To test the hypothesis that milk and/or dairy affects the components of the metabolic syndrome and to obtain an answer on how it affects, the interventions studies that are included in this work has been performed in either healthy or diseased subjects (subjects with diabetes type 2, hypertension, overweight/obesity). Intervention studies are standardized and different parameters are controlled to obtain as equal groups as possible (intervention versus placebo groups) except in the aspect that is tested. This results is the possible to evaluate if the treatment tested affects the risk parameters for the disease and which mechanism that supports such effect. However, there are several limitations with intervention studies e.g they are often performed on small groups (not always representative for a whole population), the intervention period is limited, and it is also often a low compliance to the study diets.

The metabolic syndrome in total

It is difficult to conclude the effect of milk and dairy intake on the metabolic syndrome in total. Because of the magnitude of the metabolic syndrome, comprising may factors that can have effect on the outcome of the disease, it is complicated to drawn a general conclusion. There are six cross-sectional studies included in this work that investigate the relation between milk and dairy intake and the metabolic syndrome. A majority of these studies show an inverse relationship between milk and dairy intake and the metabolic syndrome. Because of the design of these studies, a temporal relationship between milk consumption and the metabolic syndrome cannot be shown. In addition, it has been shown by Pereira et al. (2002) that low-fat dairy intake might protect against diabetes type 2 in overweight men. This study, the only one with longitudinal design, strengthens the evidence for a positive effect of milk and dairy on obesity and the metabolic syndrome.

Insulin resistance and diabetes type 2

Milk of low-fat content might protect from the development of insulin resistance and diabetes type 2 and this has been shown in several epidemiological studies. The observational data, reviewed on milk and dairy and insulin resistance and diabetes type 2, consist of two prospective cohorts studies and two cross-sectional studies. Choi et al. (2005) and Liu et al. (2006) are two studies with longitudinal design, with a large sample size. It can be concluded from these studies, that low-fat milk products might have a protective effect on the development of insulin resistance and diabetes type 2. The inverse association between low-fat dairy products and insulin resistance and diabetes type 2 seen in these studies are convincing.
Intervention studies performed within this area show that the whey fraction of milk containing branched chain amino acids has insulinogenic properties. These amino acids have been shown to stimulate postprandial insulin secretion that lowers blood glucose concentrations. There are five acute randomized controlled trials performed in healthy subjects studying glucose and insulin response after intake of whey or individual amino acids. All show positive effect on postprandial glucose control after ingestion of whey. The doses amino acids tested by Nilsson et al. (2007) resulting in 18 g of whey and it corresponds to approximately 1,1L of milk.

One study is performed on subjects with diabetes type 2, with a reduced insulin secretion, strengthens the positive role of whey in glucose and insulin control. Present intervention studies are all acute studies, and long-term, well-performed randomized controlled trials are lacking. To be able to comment on longer-term study effect of milk, whey and specific amino acids on the glucose and insulin response such studies need to be done. The acute studies suggests that a diet with a supplement branched amino acids might be useful to facilitate blood glucose control in subjects with diabetes type 2 by lowering both the 24-h glucose response and HbA1c. Researchers performing studies suggests that it might also be possible to postpone the introduction of medical treatment of diabetes type 2 patients in an early stage of the disease, if a whey supplement is introduced to the diet. Despite these beneficial effects of a whey supplement in a diet in subjects with diabetes type 2, it is not yet clarified how elevated insulin levels produced by a whey supplement affect health.

In general, a high concentration of insulin stimulates the triglyceride synthesis and fat storage in adipose tissue. This state might in turn lead to an accumulation of ectopic fat with unhealthy consequence.

**Hypertension**

**Milk and dairy intake and hypertension**

Low-fat milk and dairy have been shown to have blood pressure lowering effect in five observational studies. Of these studies, two were cohort studies and three were of cross-sectional design.

The cohort studies by Alonso et al. (2009) and Engebrink et al. (2009) were performed on subjects free of hypertension at baseline and the development of hypertension was studied prospectively. These studies show that intake of low-fat dairy products are inversely associated with blood pressure. The cross-sectional studies have also found an inverse association between milk and dairy intake and hypertension, but a conclusion of the effects seen over time cannot be drawn. However, it can be concluded that it seems to be an inverse relationship between intake of low-fat dairy products and blood pressure and that probably the calcium content of dairy might have a positive effect as shown by Jorde et al. (2000).

The hypothesised positive effect of low-fat dairy products on hypertension seen in observational studies is in concordance with results from intervention studies performed in the field. The randomized control trial performed by Appel et al. (1997) show that a diet rich in fruit, vegetables and low-fat dairy products (DASH-diet) lowers blood pressure. Hilpert et al. (2009), obtain similar results and suggests that the association seen is related to the calcium content of dairy products. In summary, the intervention studies support the inverse association seen in observational studies between low-fat dairy product intake and hypertension.

**Milk tripeptides and hypertension**

There are two meta-analysis performed on the effect of lactotripeptides on hypertension, Pripp. (2008) and Xu et al. (2008) provide evidence that milk peptides have an antihypertensive effect. However, the effect seen can only be attributed to milk products fermented with Lactobacillus Helveticus. Fermentation with Lactobacillus Helveticus
produces the tripeptides IPP and VPP, which have ACE-inhibiting effect and lowers blood pressure. In contrast to this, there are other randomized controlled trials performed on the effects of lactotripeptides that does not show any beneficial effects of these peptides on blood pressure. It is important to remember that milk and dairy form part of a whole diet, and there are also a lot of nutrients that can affect the outcome of a disease. Concluding the effect of milk and dairy on hypertension there seems to be an inverse association between intake of low-fat dairy products and blood pressure.

**Overweight and obesity**

**Milk and dairy and weight**

The observational studies included in this work have been performed on either milk or dairy, or the calcium content in dairy products, linked to different outcomes such as weight change, BMI, and waist circumference. It is therefore complicated to conclude a general association between milk and dairy consumption and overweight/obesity. There are three studies of longitudinal design and three studies of cross-sectional design studying the effect of calcium and milk and dairy on weight. Two of the longitudinal studies give similar results, Rajpathak et al. (2006) found no effect of calcium and weight gain or reduction of weight over time and Vergnaud et al. (2008) concluded that calcium intake was no associated with the inverse relation seen between milk and dairy and overweight. Rosell et al. (2006), concludes that the association between intake of dairy products and weight gain differs depending of the type of dairy products consumed. Accumulated evidence from randomized clinical trials indicating that neither dairy products nor calcium supplements reliably facilitates weight loss as summarized in a review by Lanou et al. (2008) evaluated 49 clinical trials on the effect of calcium and weight loss. No evidence were found supports the hypothesis that dairy or calcium consumption alone or in conjunction with caloric restriction, results in weight or fat loss in the short or long term. Despite the above-mentioned reviewed results, there are interesting results obtained by Zemel et al. (2004). The positive results seen in this study are attributed to the calcium content of the high dairy diet, which affected fat loss positively.

**Milk and dairy and satiety**

It is well known that proteins provide a more pronounced satiation compared to carbohydrates. Four acute intervention studies have been performed to investigate the effect of whey proteins and satiety. In the case of milk and dairy products, whey proteins are more sattetating than other proteins. Dove et al. (2009) demonstrate this effect when skim milk was compared to a fruit drink. The intake of skim milk leads to an increase in satiety as well as a reduced energy intake in a subsequent meal. Veldhorst et al. (2009) and Nieuwenhuizen et al. (2008) test the effect of alfa-lactalbumin on satiety and energy intake. Alfa-lactalbumin gives a more prolonged satiating effect, as well as a lower energy intake in the subsequent meal compared to other proteins tested. However, these studies are all performed on healthy normal-weight subject. In summary, it is of interest to see if the results seen in normal-weight subjects can be applied to overweight subjects, and to further study if, alfa-lactalbumin could facilitate weight loss in the longer run.

In summary, the effect of milk and dairy intake on the metabolic syndrome have been investigated in both observational and intervention studies. However, studies of cross-sectional design stand for the majority of the evidence in the field. The strengths of evidence obtained by cross-sectional studies are of poorer quality when it comes to translating results.
into risk reduction. This results in the difficulty to generally conclude the effect of milk and dairy on the metabolic syndrome.
Finally, what are the positive effects of milk and dairy intake and how can they act beneficially on separate components of the metabolic syndrome?

Whey proteins seems promising in the control of glucose and promoting insulinaemia. But the tested doses that have been shown to have insulinogenic properties cannot be obtained by the recommended intake of milk and dairy products. Low-fat dairy products as part of a healthy and well-balanced diet (DASH-diet) rich in fruits and vegetables have promising antihypertensive effects. The amount of dairy included in the DASH-diet is in concordance with the amount of milk and dairy intake that the Swedish National Food Administration recommends.
Finally, if this daily amount of milk and dairy consumed are composed of specific and carefully chosen milk proteins, a product with satiating characteristics might be a valuable tool in a healthy weight management.
6. Experimental part

A milk product that has beneficial effects on weight maintenance was developed at the laboratories and pilot plant of Arla Foods Fresh Dairy Products Innovation in Stockholm, Sweden. By affecting satiety it can be hypothesised that weight gain can be avoided by inducing a more pronounced satiety after intake of this milk product. Therefore a stable and healthy weight can be obtained. Specific and carefully chosen proteins are added to the product. Probiotic bacteria and fibre, two components that have beneficial effects on the gut’s microflora, are also added to the milk product. Information about the combination of ingredients included in the milk product as well as the process techniques cannot be given since its company confidential.

6.1 Materials and methods

6.1.1 Experimental part in laboratory scale

Selection of different proteins and fibre

Four different protein fractions and a whey protein concentrate were selected and the solubility, taste and texture were evaluated. The solubility of the fibre type was also tested. The amount that corresponds to 3,6g/100g protein of each protein and 4,5g/100g fibre was added to 200ml ultra high-temperature milk (UHT-milk). The test drinks were stirred for 60min and then kept refrigerated over night. A reference drink composed of 200 ml UHT-milk was included.

Test drinks:
A) Protein fraction + 200 ml UHT-milk
B) Acid and heat stable protein fraction + 200 ml UHT-milk
C) Protein hydrolysate + 200 ml UHT-milk
D) Fibre + 200 ml UHT-milk
E) Protein fraction + fibre+ 200 ml UHT-milk
F) Acid and heat stable protein fraction + fibre + 200 ml UHT-milk
G) Protein hydrolysate + fibre + 200 ml UHT-milk
H) Neutral protein + fibre + 200 ml UHT-milk
I) Whey protein concentrate + fibre + 200ml UHT-milk, Reference test

A test panel evaluated the drinks and those proteins that are approved were used in further tests. Before tasting the drinks, a visual evaluation was performed on all drinks.

Sedimentation test of calcium

Two drinks were prepared to test if calcium added to milk sediments after dilution. 40 mg calcium/100g was added to 200 ml UHT-milk to drink 1. The same amount of calcium (40 mg calcium/100g) and 4,5g/100g fibre was added to drink 2. The calcium and fibre amount were diluted and then stored refrigerated over night.
Heat treatment of test drinks
Each different protein (3.6g/100g) was diluted in 1000g of fresh milk of 1.5 % fat content. An amount of 4.5g/100g of fibre was also added to each drink. The drinks were stirred for 60 min at room temperature.
After dilution, each drink was heat treated and cooled on ice afterwards. A visual evaluation on the texture of the drinks was made after heat treatment.

Test drinks:
Test drink E containing protein fraction and fibre
Test drink F containing acid and heat stable protein and fibre
Test drink H containing neutral protein and fibre
Test drink I containing whey protein concentrate and fibre, Reference test

Microbiological tests part 1
0.1 ml of each product sample was transferred to the surface of the blood agar plates to investigate if there was any contamination. The inoculum was distributed evenly using a sterile, bent rod throughout the surface of the substrate.
When the inoculum had been absorbed into the substrate, the petri dishes were inverted and incubated at 30 °C for three days.

Inoculation with the probiotic bacteria Lactobacillus F19 and Streptococcus Thermophilus (ST20), part 1
The amount of protein (3.6/100 g) and fibre (4.5 g/100 g) was diluted in 1000 g fresh milk of 1.5 % fat content. Two preparations were made for each test drink. A reference test was also included containing 1000 g of fresh milk (drink 5). The drinks were then heat treated and stored refrigerated over night.
Test drink E was renamed and became drink 1 and 2, both contained protein fraction. Test drink I became test drink 3 and 4 and both contained whey protein concentrate as table 7 shows.

Thereafter an adequate amount of Lactobacillus F19 was added to drink 2, 4, and 5 and ST20 was added to all test drinks. For the composition of each test drink see also table 7. The test tubes were placed in a water bath. In order to obtain appropriate concentrations of ST20, the bacteria were dissolved in UHT-milk before inoculation.

Table 7. Composition of the test drinks.

<table>
<thead>
<tr>
<th>Drink</th>
<th>Bacteria 1 (ST20)</th>
<th>Bacteria 2 (F19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, Protein fraction and fibre</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>2, Protein fraction and fibre</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3, Whey protein concentrate (Ref)</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>4, Whey protein concentrate (Ref)</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>5, Milk reference</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

During fermentation after inoculation with bacteria, the bacteria will use the carbohydrates in milk and produce lactase. As a result, pH will decrease. Therefore, a small portion of each drink (100ml) was used to measure pH every 15 min during the fermentation process until pH4.5 was reached.
The bottles were stored in the refrigerator over night and then the milk product was evaluated on the taste and texture.
**Inoculation with Lactobacillus F19, ST20 and BLA, part 2**

The process of preparation before inoculation is the same as in part 1. Thereafter Lactobacillus F19 was added to all test drinks. ST20 was added to drink 1, 3, and reference A and BLA was added to drinks 2, 4 and reference B. For the composition of the test drinks see also table 8.

**Table 8. Composition of the test drinks.**

<table>
<thead>
<tr>
<th>Drink</th>
<th>Bacteria 1 (ST20)</th>
<th>Bacteria 2 (BLA)</th>
<th>Bacteria 3 (F19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, Protein fraction and fibre</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>2, Protein fraction and fibre</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>3 Whey protein concentrate (Ref)</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>4 Whey protein concentrate (Ref)</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>A Milk reference</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>B Milk reference</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

The drinks were placed in a water bath and pH was measured every 15 min until pH 4.5 was reached. All the bottles were stored refrigerated over night.

**Microbiological tests part 2**

This step was performed to investigate if the amount of bacteria inoculated at step 1 and 2 is in concordance with the amount expected after fermentation (the concentration content is confidential information). Each test drink was divided into 3 bottles of 200 ml representing day 0, 7, and 14. The bacterial growth in the drinks was detected using MRS-agar plates (deman, Rogosa, Sharpe) that are specific for lactobacillus bacteria and M17-agar plates are specific for S. Thermophilus. By plating a diluted sample of each of the test drinks, the concentration of the bacteria growth can be obtained. The tests performed on blood agar plates were performed in the same way as in microbiological tests part 1.

**6.1.3 Experimental part of the pilot plant scale**

The process performed in the laboratory scale was transformed to a process in a pilot plant scale. A standard yogurt process was performed for the milk product (Dairy Process Handbook 1995). For the composition of the test drinks see table 9.

**Following test drinks were included:**

**Table 9. Composition of the tests drinks.**

<table>
<thead>
<tr>
<th>Drink</th>
<th>Bacteria 1 (ST20)</th>
<th>Bacteria 2 (BLA)</th>
<th>Bacteria 3 (F19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 100% Protein fraction and fibre</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>2, 100% Protein fraction and fibre</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>3, Mix of 50/50 % of whey protein concentrate and protein fraction and fibre</td>
<td>x</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>4, Mix of 50/50 % of whey protein concentrate and protein fraction and fibre</td>
<td></td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
The test product 3 and 4 was a mix of 50/50 % whey protein concentrate and protein fraction plus fibre, and was used as a reference. This because it was important to study if this ratio amount of proteins, behaved differently to the product containing 100% of the protein fraction, drink 1 and 2.

**Following process was performed:**

Mix of proteins and fibre in 1,5 % fresh milk  
↓  
Homogenisation  
↓  
Heat treatment  
↓  
Cooling  
↓  
Inoculation with F19, ST20, BLA  
↓  
Fermentation in tank  
↓  
Cooling 1 → Dispense to small cans  
Cooling 2 → Dispense to small cans  
Back pressure → Dispense to small cans  
↓  
Cooling  
↓  
Refrigerated storage  

Dispense to small cans to make set-yogurt  
↓  
Fermentation in can  
↓  
Cooling  
↓  
Refrigerated storage

All the ingredients were mixed with fresh milk of 1,5% fat content. After the milk was homogenised, heat treated, and cooled to an adequately temperature for inoculation with bacteria. Thereafter, two different kinds of fermentations took place.
The first type of fermentation started with using small cans, which were filled with the milk product to make set-yogurt (see process schedule). It was performed to investigate if this process was possible to do and what kind of product that could be obtained. The product samples processed to set-yogurt were after fermentation cooled and stored refrigerated.

The other kind of fermentation was done in the tanks and was also tested with the same aim as the fermentation in the can.
Fermentation was stopped when pH 4,5 was reached (both in the cans and in the tank) as in the laboratory experiment. After fermentation, these product samples were cooled at two different temperatures to investigate the texture and appearance of the product after these technical steps.
The product was also processed with back-pressure to investigate if it is possible to produce a fluid drink milk product.
All of the test products obtained from the pilot experiment were stored refrigerated.
The same microbiological tests were made as in the *microbiological test part 2* in the laboratory scale to investigate bacterial growth.
6.2 RESULTS

6.2.1 Experimental part in laboratory scale

Selection of different proteins
No visual sedimentation was seen on any of the drinks, the proteins and fibre were well dissolved in milk. The results from the tests are presented in table 10.

Taste evaluation of the test drinks:

Table 10. Results from the taste test of the different test drinks.

<table>
<thead>
<tr>
<th>Test drinks</th>
<th>Taste</th>
<th>Oral feeling</th>
<th>Approved Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, Protein fraction</td>
<td>Slightly metallic Taste can probably be hidden with addition of fruit taste</td>
<td>Smooth and round</td>
<td>Yes</td>
</tr>
<tr>
<td>B, Acid and heat stable protein fraction</td>
<td>Sour and &quot;sticky&quot; bitter. Doubtful if taste can be hidden.</td>
<td>Smooth and round</td>
<td>Yes, needs more tests</td>
</tr>
<tr>
<td>C, Protein hydrolysate</td>
<td>Bitter, sour, horrible taste!</td>
<td>Sticky</td>
<td>No</td>
</tr>
<tr>
<td>D, Fibre</td>
<td>Sweet, mellow, caramel taste</td>
<td>Smooth and round</td>
<td>Yes</td>
</tr>
<tr>
<td>E, Protein fraction and fibre</td>
<td>&quot;Powder&quot; taste</td>
<td>Smooth</td>
<td>Yes</td>
</tr>
<tr>
<td>F, Acid and heat stable protein fraction and fibre</td>
<td>Sour and &quot;sticky&quot; bitter. Doubtful if taste can be hidden.</td>
<td>Smooth</td>
<td>Yes, need more tests</td>
</tr>
<tr>
<td>G, Protein hydrolysate and fibre</td>
<td>Bitter, sour, horrible taste!</td>
<td>Smooth</td>
<td>No</td>
</tr>
<tr>
<td>H, Neutral protein and fibre</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
<tr>
<td>I, Whey protein concentrate and fibre, Reference</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Following test drinks were approved; Drink E, F, H, and I were approved and were used in further experiments see also table 12. Drink G and F were not tasted because their characteristics did not correspond to what could be useful in a future milk product.
Sedimentation test of calcium

When calcium was added to the milk product an obvious sedimentation was seen (table 5). Therefore, calcium cannot be added to the products to obtain 40mg/100g calcium because of its sedimentation in the milk drink. It could be possible to add carragenan to the product to avoid this sedimentation of calcium, like the calcium-enriched milk Arla has. However, carrageenan has to be declared as an additive and consumers can choose not to buy the product because of this reason. In conclusion, calcium is not added to the product.

Table 11. Sedimentation tests.

<table>
<thead>
<tr>
<th>Drink test</th>
<th>Sedimentation Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Calcium + 200 ml UHT-milk</td>
<td>Yes</td>
</tr>
<tr>
<td>2) Calcium + fibre+ 200 ml UHT-milk</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Heat treatment of test drinks

The results after heat treatment of the test drinks for visual evaluation are presented in table 12.

Table 12. Visual evaluation of the selected test drinks

<table>
<thead>
<tr>
<th>Drink</th>
<th>Visual evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>E; Protein fraction and fibre</td>
<td>Clear solution with no visible particles</td>
</tr>
<tr>
<td>F; Acid and heat stable protein and fibre</td>
<td>Coagulation of proteins. The drink has been divided in a water phase and a thick phase.</td>
</tr>
<tr>
<td>H; Neutral protein and fibre</td>
<td>Little coagulation, looks like “filmjölk”</td>
</tr>
<tr>
<td>I; Whey protein concentrate and fibre. Reference drink</td>
<td>Clear solution with no visible particles</td>
</tr>
</tbody>
</table>

Taste evaluation:

Drink F and H were not tasted because of their appearance and were therefore discarded from further tests.
Drink E and I tasted like “välling” and were used for inoculation with probiotic bacteria.

Microbiological tests

No contamination was found in the microbiological tests.
Inoculation with the probiotic bacteria Lactobacillus F19 and Streptococcus Thermophilus (ST20)

Figure 4. Fermentation diagram for each test drink.

![Fermentation with Lactobacillus F19 and ST20](image)

Serie 1: Test drink 1  
Serie 2: Test drink 2  
Serie 3: Test drink 3  
Serie 4: Test drink 4  
Serie 5: Milk reference

The results on fermentation after inoculation with probiotic bacteria are presented in figure 4. For the composition of each test drink see also table 7. In general, drink 2 and 4 reached pH 4.5 after nearly 6.5-7.5 hours. These drinks contained both Lactobacillus F19 and Streptococcus Thermophilus that acted synergistically with each other. Drink 1 that only contained Streptococcus Thermophilus, the fermentation went very slow. It is possible that something in the drink could have inhibited fermentation.

The taste evaluation of the test drinks are presented in table 13.

<table>
<thead>
<tr>
<th>Test drinks</th>
<th>Taste</th>
<th>Oral feeling</th>
<th>Texture</th>
<th>Approved Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, Protein fraction and fibre</td>
<td>Mild sour taste, mellow caramel</td>
<td>Smooth</td>
<td>Thick, coagulation to maximum</td>
<td>Yes</td>
</tr>
<tr>
<td>2, Protein fraction and fibre</td>
<td>Mild yogurt taste</td>
<td>Smooth</td>
<td>Very thick</td>
<td>Yes</td>
</tr>
<tr>
<td>3, Whey protein concentrate (Ref)</td>
<td>More sour than drink 1</td>
<td>Smooth</td>
<td>Thick</td>
<td>Yes</td>
</tr>
<tr>
<td>4, Whey protein concentrate (Ref)</td>
<td>More sour than drink 1</td>
<td>Smooth</td>
<td>Thick</td>
<td>Yes</td>
</tr>
<tr>
<td>5, Milk reference</td>
<td>Mild sour taste</td>
<td>Smooth and milk</td>
<td>Fluid like a yogurt</td>
<td></td>
</tr>
</tbody>
</table>

All of the drinks had a smooth taste and nice appearance. Drink 3 and 4 tasted more sourly than drink 1. This taste could be hidden with additions of berries or other taste additives.
Inoculation with Lactobacillus F19, ST20 and BLA, part 2

Figure 5. Fermentation diagram for each drink

![Fermentation diagram with Lactobacillus F19, ST20 and BLA](image)

Serie 1: Test drink 1
Serie 2: Test drink 2
Serie 3: Test drink 3
Serie 4: Test drink 4
Serie A: Reference drink A
Serie B: Reference drink B

The results after inoculation with probiotic bacteria are presented in figure 5. For the composition of the test drinks see table 8.
The fermentation went fastest in the drinks containing both BLA and Lactobacillus F19, which reached pH 4,5 after approximately 5,5 hours. The slowest fermentation was in drink A, containing ST20 and Lactobacillus F19.

Microbiological tests part 2
MRS plates that are specific for Lactobacillus F19 and BLA showed that there was a very low degree of bacterial growth in the product. This was not expected. It might be an unknown factor that is inhibiting the growth of the bacteria.

M17 plates were used because of its specificity for Streptococcus Thermophilus and the results were the same as in the MRS plates. The bacterial growth was not as expected and it might be an unknown factor inhibiting the bacteria.

The blood agar plates showed no contamination after the testing period of 0, 7, and 14 days.
6.2.2 Experimental part in pilot plant scale

The experiment was stopped when pH reached 4.8 because pH did not go below this value. The product was extremely thick and compact at that time. Because of this, it was not possible to perform the different cooling stages and back-pressure stage. However, it was possible to obtain milk product directly from the tank without any process to perform microbiological test to investigate the bacterial growth status. The microbiological tests showed a lower bacterial growth in all tested drinks, inoculated with bacteria, than expected.

6.3 Summary and discussion of the experimental part

Experimental part of the laboratory scale
The experiment performed in the laboratory scale gave information of what kind of proteins and protein fractions that could be useful in a milk product in combination with a fibre and probiotic bacteria. The proteins and protein fraction that were tested further had the best taste and appearance. The next step to go was to investigate how these ingredients behave in a pilot plant experiment. In addition, an evaluation of what kind of milk product that could be produced with different process techniques is also an important outcome of a pilot plant experiment. However, it is unknown why the bacterial growth is not as expected. It might be the fact that something is lacking in the product that affects the growth of the bacteria or that an unknown factor is inhibiting their growth.

Experimental part of the pilot plant scale
The experiment performed in the pilot plant scale gave useful information of how the combinations of ingredients behaved in the process and if it could be a future milk product. It can be concluded that the product with the combination and amounts of ingredients tested, cannot be processed. To be able to produce the milk product with these ingredients, a change in the process schedule must be done. It could be hypothesised that a different ratio of proteins than the one tested might behave differently. If so, the milk product could be processed with a standard yogurt process technique. The next step to go is to investigate further what ratio of protein amounts could be used because the drink containing 50/50 was not processable. It is very important to obtain a specific amount of probiotic bacteria in the products so it can have beneficial effects on the gut’s microflora. Therefore, it is of high interest to study why the probiotic bacteria are not growing as expected. As mentioned early, it might be the fact that something is inhibiting their growth when these ingredients are blended together. Further investigation on these aspects is needed for the development of the milk product that might have beneficial effects on weight control.
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Mjölkfrämjandet: http://www.mjolkframjandet.se/faktabank/naringsfakta/vitaminer_och_mineraler/mjolk_innehaller_18_av_22_naringsamnen_vi_behover_varje_dag/


Appendix

1. Epidemiology and study designs
Epidemiology is the study of factors seen in populations that affects health and illness, and serves as the foundation and logic of interventions made in the interest of public health and preventive medicine. It is considered a cornerstone methodology of public health research, and is highly regarded in evidence-based medicine for identifying risk factors which can lead to disease, and for determining optimal treatment approaches to clinical practice. There are different types of studies to perform when searching for a link between risk factors and outcome of a disease; these studies are described further below. (Ahlbom et al. 2006).

The strengths of evidence of the epidemiological studies differ from each other in the following hierarchal scale:

Figure 1. Strength of evidence of epidemiological studies

<table>
<thead>
<tr>
<th>RCT of disease outcome</th>
<th>RCT of physiologic measures/ risk factors</th>
<th>Retrospective case-control studies</th>
<th>Animal studies</th>
<th>Ecological studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prospective cohorts of disease outcomes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Best evidence

Poor evidence

(Adapted and modified from Micha et. al. 2010)

1.1 Observational studies

1.1.2 Cohort study
A cohort study is often performed to obtain evidence to try to refute the existence of a suspected association between cause and effect; failure to refute a hypothesis strengthens confidence in it. Before the appearance of the disease under investigation, the cohort is identified. The study population of a cohort study is observed over a period of time to determine the frequency of new incidence of the studied disease among them. Therefore, the cohort can not be defined as a group of subjects who already have the disease. A cohort study can either be of retrospective or of prospective (longitudinal) type. A retrospective cohort is performed when data of the study subjects is already collected. The results from a restrospective cohort are summarized with odds ratio. In prospective cohorts, the study group is defined before the study is performed and the results are expressed as the relative risk. (Ahlbom et al. 2006)

1.1.3 Case-control study
A case-control study is performed to identify factors/risk factors that may contribute to a medical condition by comparing subjects who have the condition of interest (cases), with patients who do not have the condition but are otherwise similar (controls). Case-control studies starts with subject who are diseased or have other medical conditions. To collect information about the past and investigate if there are characteristics of these subjects that differ from those who do not have the disease, is the major challenge of a case-controll study.
This type of study does not give any indication of the absolute risk of the factor studied. Instead, it gives information about a certain behavior that may be associated with an increased risk as compared with the control group. (Ahlbom et al. 2006)

1.1.4 Cross-sectional study
Cross-sectional studies can be performed to give an overview of the frequency and characteristics of a disease in a population at a particular point in time. The results from a cross-sectional study can be used to assess the prevalence of the studied disease or other chronic conditions in a population. A disadvantage of this type of study is that since the exposure and the disease status are measured at the same point in time, it is difficult and complicated to distinguish whether the exposure preceded or followed the disease. (Ahlbom et al. 2006)

1.2 Intervention studies

1.2.1 Randomized controlled trials (RCT)
A randomized controlled trial is a type of cohort study where the research leader randomly divides the exposure tested (a diet or a treatment for example) among the study population, which is then compared with a placebo group. The aim of these studies is to evaluate if and how a treatment or intervention affects the recovery, illness or the consequences followed by a disease. The strength in a randomized study is that all of the risk factors beyond the studied exposure, which eventually could affect the outcome, are randomly divided. This leads to the minimized risk of having confounding factors that can affect the results. (Ahlbom et al. 2006)
2. Effects of milk and dairy intake on the metabolic syndrome in total

2.1 Epidemiology

2.1.1 Evidence from observational studies

In 2002, Pereira et al. published results from the prospective CARDIA study material investigating the relationship between dairy consumption and obesity and insulin resistance syndrome. In total, 3157 white and black young overweight and normal weight adults (18-30 years old) in the U.S participated in the study with a follow-up period of 10 years. A quantitative food frequency questionnaire was used to estimate relative intake per week for each food item (portion size not defined). The most frequently consumed dairy product at baseline was milk and milk drinks, followed by butter, cream, and cheeses. The result from this study show an inverse association between frequency of dairy intake and development of obesity, abnormal glucose homeostasis, elevated blood pressure and dyslipidaemia among overweight subjects (BMI ≥ 25) at baseline, but not among normal weight subjects. A suggested explanation for the loss of positive effect of dairy intake among normal weight subjects is that these subjects have a different lifestyle and eating habits or perhaps genetic predisposition. The adjusted odds ratio of developing metabolic syndrome were 72% lower among overweight individuals in the highest category of dairy consumption (>5 times/weeks) compared with the lowest category of dairy consumption (<1.5 times/week). Each additional daily occasion of dairy consumption was associated with 21% lower odds ratio of suffering from insulin resistance syndrome. These associations were similar for overweight men and women and independent of race. Results were adjusted for confounding lifestyle and dietary factors and authors suggest therefore that the association is entirely explained by the dairy intake.

In summary, authors conclude that dairy consumption has a strong inverse association with insulin resistance syndrome among overweight adults and may reduce risk of type 2 diabetes and cardiovascular disease. (Pereira et al. 2002)

Snijder and colleagues (2007) aim to evaluate the association of dairy intake and body weight and other components of the metabolic syndrome in a cross-sectional design with data from the Hoorn study. The number of study participants were 1896 men and women (50-75 years). To assess average food intake a 92-item semi quantitative food frequency questionnaire was used. The serving sizes for dairy product was defined as 150 g liquid products and 20 g solid products. Total dairy consumption was divided in two categories, low-fat (≤2% fat) or high-fat dairy (>2% fat). The median consumption of total dairy products was 4,1 servings per day. The results from this study show that, after adjustment for age and sex, total intake of dairy was borderline significantly inversely associated with systolic and diastolic blood pressure and triacylglycerols concentrations but not with BMI or any of the other components of the metabolic syndrome. When high-fat and low-fat products were distinguished, consumption of high-fat dairy was inversely associated with BMI, waist circumference, triacylglycerol, and insulin and significantly positively associated with HDL-cholesterol concentrations. In contrast, low-fat dairy was significantly positively associated with BMI, waist circumference and fasting glucose concentrations.

The authors conclude that there is a modest inverse association with several dairy products with blood pressure but not with BMI or other components of the metabolic syndrome. (Snijder et al. 2007)
Elwood and colleagues (2007) investigated the association between milk or dairy consumption and the metabolic syndrome prevalence in the Caerphilly cohort of 2512 middle-aged (45-59 years) men. Food consumption was assessed by a semi-quantitative questionnaire including the quantity of milk drunk each day. At five-year intervals, the study participants where re-examined and follow-up was continued for 10 years.

The results from this study show a significant negative relationship between milk consumption and the presence of the metabolic syndrome. Men who drank one pint (approximately 0.5L) of milk or more per day had an adjusted odds ratio for developing the metabolic syndrome of 0.38 relative to men who stated that they drank little or no milk.

The authors conclude that the consumption of milk and dairy products is associated with a markedly reduced prevalence of the metabolic syndrome. (Elwood et al. 2007)

Beydoun and colleagues (2008) performed a cross-sectional study to examine the association between consumption of dairy products and their related nutrients with obesity, central obesity, and metabolic syndrome. The study population consisted of 17 061 men and women from the National health and nutrition examination survey 1999-2004. The amount of food-consumed daily was estimated by 24-hour recall interview. Dairy composed one group containing milk of various fat content, cheese and yogurt. The mean dairy intake was estimated to 243 g/day.

On average each serving of dairy products increased the risk of metabolic syndrome by 8% among men. Whole milk was weakly and negatively associated with the prevalence of central obesity, whereas low-fat milk had the opposite effect. Each additional serving of yogurt was associated on average with a 2 to 2,5-fold reduction in the risk of developing obesity, central obesity and the metabolic syndrome. When dairy consumption and related nutrients were examined in relation to individual metabolic outcome as continuous variables, the results show that among all subjects, and among men in particular, yogurt was associated with better metabolic outcomes, reduced BMI, waist circumference, systolic blood pressure and fasting glucose. In addition, reduced total number of disturbances in the whole sample, and increased HDL-cholesterol level among women.

The authors suggest that the health effects of dairy products and related nutrients are complex and may not be uniform across the population, at least for obesity and related metabolic disorders. (Beydoun et al. 2008)

Azadbakht et al. (2005) investigate the relation between dairy consumption and the metabolic syndrome in a cross-sectional study of 827 slightly overweight Iranian adults (BMI 25-27 kg/m²), aged 18-74 years.

The dairy intake was estimated by a semiquantitative food frequency questionnaire. Cut-off for quartiles of dairy intake was calculated, and subjects were categorized according to the quartiles. The reported mean dairy intake was 0,7 serving/day for milk, 1,06 serving/day for yogurt and 0,9 serving/day for cheese.

Results from this study show that subjects in the fourth quartile of dairy intake (≥2,7 servings per day) had a lower BMI than those in the third lower quartile (1,8 to <2,7 servings per day). The frequency of metabolic syndrome and its components was the highest in the lowest quartile (< 1,0 servings per day) of dairy intake. Subjects in the fourth quartile of dairy intake had significantly lower mean waist circumference that subjects in the first quartile. Furthermore, subjects in the fourth quartile had significantly lower mean systolic and diastolic blood pressure that did subjects in quartile 1 and 2.

A higher intake of dairy was associated with a healthier diet, and subject in the highest quartile also consumed more fruit and vegetables and less meat than did subjects in the lowest quartile.
The authors conclude that dairy consumption is inversely related to the risk of having the metabolic syndrome. (Azadbakht et al. 2005)

Liu and colleagues (2005) examine whether and to what extent intakes of calcium and vitamin D are related to the metabolic syndrome in middle-aged or elderly women. The study population of this cross-sectional study consisted of 10,066 from the Women’s health study. A semi-quantitative food frequency questionnaire was used to estimate dietary intake. Total calcium and vitamin D intake were calculated from both dietary and supplemental sources. The mean daily intake of individual dairy items were combined to compute dairy intake. The median intake for total calcium was 857 mg/day (88% from diet and 12% from supplements) and for vitamin D 266 IU equivalent to 6.65 µg (84% from diet and 16% from supplements). Results show that the prevalence of each of the five components of the metabolic syndrome was lower in women in the highest quintile of calcium and vitamin D intake (prevalence 0.6 for total calcium and vitamin D intake) compared to women in the lowest quintile (1.1 for total calcium and 1.2 for total vitamin D intake). Hypertriglyceridemia was not associated with total intakes of calcium and vitamin D and incident of diabetes type 2 was not associated with dietary vitamin D intake. In the age- and total calorie-adjusted model, total calcium intake was significantly inversely associated with the prevalence of metabolic syndrome. In contrast, a significant inverse association was consistently evident only for dietary vitamin D and the metabolic syndrome, but this association appeared to be entirely explained by adding total calcium intake. There were trends for lower prevalence of the metabolic syndrome associated with total dairy products, high-fat dairy products, low-fat dairy products and total milk intake.

In conclusion, a high calcium intake and dairy product consumption are associated with a lower prevalence of the metabolic syndrome in middle-aged and elderly women. However, no significant inverse association between vitamin D intake and the metabolic syndrome was shown. (Liu et al. 2005)

Lawlor et al. (2005) examine the association of milk consumption with insulin resistance and the metabolic syndrome in a cross-sectional set up. Data from the British Women’s Heart and Healthy study were used comprising 4024 middle-aged women (60-79 years old) randomly selected from primary care centres. Women reported in a self-completed food frequency questionnaire whether they drank milk and the type of milk they drank. Insulin resistance was estimated according to the homeostasis model assessment (HOMA). Measurements of components of the metabolic syndrome were obtained using standard procedures.

Results from this study show that 111 women of the 4024 reported never drinking milk. The women who never drank milk had lower mean HOMA scores, triglycerides levels and BMIs and higher HDL-levels than those who drank milk; they were also less likely to have diabetes type 2 or the metabolic syndrome. The aged-adjusted odds ratio for the metabolic syndrome comparing none milk drinkers with milk drinkers was 0.55.

In conclusion, the authors suggest that individuals who do not drink milk may be protected against insulin resistance and the metabolic syndrome. (Lawlor et al. 2005)
3. Effects of milk and dairy intake on insulin resistance and diabetes type 2

3.1 Epidemiology

3.1.1 Evidence from observational studies

The Health Professionals Follow-up study is a prospective cohort study involving 41,254 middle-aged men (40-75 years of age) followed for 12 years onwards. Choi and colleagues (2006) aim to evaluate the relation between dairy intake and incident of type 2 diabetes in the above mentioned material. To estimate dietary intake a semi-quantitative food frequency questionnaire was repeatedly used throughout the study period.

The results show that after adjusting for age and BMI, physical activity, and dietary factors, the relative risk for men in the top quintile of total dairy intake (≥ 2.9 servings/day) was 0.77 compared with those in the lowest quintile of dairy intake (< 0.9 servings/day). Each increase in dairy servings per day was associated with a 9% lower risk of diabetes type 2. When the association was examined stratified by fat content of dairy consumption, the significant inverse association was primarily limited to low-fat dairy products.

In conclusion, authors suggest that dietary patterns characterized by higher dairy intake, especially low-fat dairy intake, may lower the risk of type 2 diabetes. (Choi et al. 2005)

Liu et al. (2006) aim to prospectively examine the association between intake of dairy foods and calcium and incidence of type 2 diabetes in 37,183 women without history of diabetes, cardiovascular disease and/or cancer, followed for 10 years. Dietary intake was assessed by a semi-quantitative food frequency questionnaire. Dairy products were classified and grouped according to fat content. Low fat dairy products included skim or low fat milk, sherbet, yogurt, and cottage/ricotta cheese. High fat dairy food, included whole milk, cream, sour cream, ice cream, cream cheese and other cheese. Total dairy products included all of the above mentioned.

Results from this study show that after adjusting for age, treatment assignment, and total energy intake the relative risk (RR) for type 2 diabetes among women in the highest quintile of total dairy intake (2.9 servings/day) was 0.79 compared with those in the lowest quintile of dairy intake (< 0.85 servings/day). Each serving-per-day increase in dairy intake was associated with a 4% lower risk for type 2 diabetes. The inverse association with type 2 diabetes appeared to be mainly attributed to low-fat dairy intake with a relative risk of 0.64 in the highest quintile of dairy intake compared with the lowest quintile for low-fat dairy intake, while the same comparison for high-fat dairy intake results in a relative risk of 1.08.

Additional adjustment for other factors including vitamin D and calcium did not attenuate this association.

In conclusion, a moderate inverse association between dairy consumption, especially low-fat dairy consumption, and diabetes type 2 was seen. (Liu et al. 2006)

Hirschler and colleagues (2009) perform a cross-sectional study on 365 10-year old Argentinean children. The mothers were asked about their children’s lifestyle behaviours and paediatricians completed questionnaires. A 5-level index ranked participants according to daily consumption of glasses of milk, vegetables or fresh fruits, sweetened beverage, and number of hours of TV viewing was used (1, 2, 3, 4, 5 daily). HOMA-IR (homeostasis model of assessment of insulin resistance), a method to quantify insulin resistance and beta-cell function, was used. The reported median daily intake of milk was 2 servings/day and the great majority reported drinking full fat milk (serving size not defined).
Results show that multiple regression analysis with HOMA-IR as a dependent variable show a negative association with milk consumption.

In conclusion, the authors suggest that increased milk consumption is associated with greater insulin sensitivity that in turn might reduce the risk of diabetes type 2. (Hirschler et al. 2009)

In contrast to the above, one study show an association between milk consumption and insulin resistance and diabetes type 2.

A brief report made on data from the ATTICA study aim to cross-sectionally evaluate the association of various food groups with indexes of glycemic control in adults (1514 men and 1528 women) with or without diabetes type 2 during 2001-2002. The dietary data was based on a validated food-frequency questionnaire and all participants were asked to report the average intake (per week or day) of several food items that they consumed during the past 12 months. The mean dairy product intake was estimated to 1-2 servings per day (serving size not defined).

The results from this study show that whole milk consumption do not correlate with indexes of glycemic control in either diabetic or non diabetic subjects. Multiple regression analysis showed a strong positive association between whole milk consumption and blood glucose and insulin, but not insulin sensitivity in diabetic subjects.

As a result, authors suggest that increased consumption of whole milk products is associated with insulin resistance. (Papakonstantinou E et al. 2005)

### 3.1.2 Evidence from intervention studies

A study where casein and whey proteins are compared on their speed of absorption show that whey proteins absorbs faster than caseins. A theory to why this occurs is because whey contains soluble proteins that exits the stomach faster and are therefore digested and absorbed more rapidly, which in turn result in a more pronounced postprandial plasma amino acid response. Caseins on the other hand, clots in the stomach due to their insoluble properties and this results in a delayed gastric emptying and a slower absorption of amino acids into the blood stream. The speed of absorption and release of amino acids to the blood stream is important due to the insulinogenic properties shown for some amino acids. (Nilsson et al. 2006, Boirie et al. 1997, Frid et al. 005)

Nilsson and colleagues (2004) perform an intervention study where they investigate the effect of common dietary sources of animal or vegetable proteins on concentrations of postprandial blood glucose, insulin, amino acids and incretin hormones. Twelve healthy subjects with normal BMI are served test meals at breakfast consisting of reconstituted milk, cheese, whey, cod or wheat gluten with equivalent amount of lactose. A carbohydrate load of white-wheat bread are used as a reference meal. Whey proteins and roller-dried skimmed milk are tested as a drink, whereas casein is administrated in the form of cheese. Blood samples were collected to follow postprandial glucose-and insulin responses for 120 minutes post meals.

Results show that the milk powder and whey drinks give lower postprandial glucose responses, expressed as area under the curve (AUC) than did the reference. The insulin response registered after whey intake is significantly higher than from all other test meals.

In conclusion, these results suggest that the insulinotropic component may be caused by the soluble whey proteins. Branched chain amino acids are produced during digestion of whey proteins. Authors suggest that the insulinogenic response of whey is contributed to the high-branched amino acids, where leucine has the most potent effect on insulin secretion but also
isoleucine, valine, lysine and threonine have a similar effect. A suggested mechanism of how these amino acids affect insulin levels could be an activation of the incretin system, where two studied hormones of the incretin system are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) (Nilsson et al. 2004). These hormones increase the amount of insulin release from the beta cells in pancreas after a meal, even before the levels of blood glucose become elevated. In addition, GIP and GLP-1 can also inhibit glucagon release from the alpha cells resulting in an inhibited glucose secretion. (Widmaier et al. 2006)

In a subsequent study by the same research group Nilsson et al. (2007) aim to determine the acute effect of specific amino acids on the postprandial insulin response in twelve healthy subjects with normal BMI. The postprandial responses of blood glucose, serum insulin, were evaluated after ingestion of drinks containing equicaloried amounts of glucose with or without addition of free amino acids at breakfast after an overnight fast. Leucine, isoleucine, valine, threonine, and lysine were selected because of their predominant appearance in postprandial blood after whey ingestion, and they were included in amounts corresponding to their contents in whey. A glucose-equivalent whey protein drink was included as a reference. Results show that the blood glucose responses after ingestion of the whey drink and a drink with a mix of whey proteins (2.2g leucine, 1.1g isoleucine, 1.1g valine, 1.6g lysine and 1.4g threonine) were significantly lower than the blood glucose response after ingestion of the reference drink (glucose). Similar results were obtained for postprandial insulin responses; the whey drink and the drink containing a mix of amino acids were found to induce significantly higher insulin AUCs than did the reference drink (glucose).

In conclusion, leucine, isoleucine, valine, lysine and threonine are likely to act as insulin secretagogues after consumption of a whey drink. Furthermore, in this study of branched chain amino acids present in whey, leucine was shown to have the most potent insulin stimulating effect. (Nilsson M et al. 2007)

Liljeberg et al. (2001) performed an acute study to evaluate the impact of milk added to a high-glycemic index (GI) white bread meal vs a low-GI spaghetti meal respectively, on postprandial glucose and insulin responses in 10 healthy subjects with normal BMI. The high-GI test meals were based on white bread and the low-GI test meals where based on spaghetti. These were served with either 200 ml or 400 ml of milk respectively or 400 ml of water. The subjects were served the meals at breakfast in random order after an overnight fast. Blood samples collected on each participant and blood glucose and insulin levels were determined throughout the testing day of 2 hours. Results show that there was no difference in postprandial glucose area under the curve with and without added milk in the case of high-GI bread meals. Same results were obtained when milk at 200 ml and 400 ml was added to the spaghetti meal. However, a higher insulin response was seen with both the high-GI meal with 400 ml and the low-GI meal with 200 ml and 400 ml milk compared to the control meal.

In conclusion, the addition of milk to a low-GI spaghetti meal may significantly increase the postprandial insulinaemia. (Liljeberg et al. 2001)

Pedersen et al. (2009) conducted an acute randomized controlled study to determine the impact of a glycemic index lowering peptide ingredient (GILP) on postprandial glycemia. This peptide ingredient consisted of a mix of whey peptides and intact whey protein containing a high concentration of branched amino acids. Ten healthy slightly overweight subjects (3 males and 7 females) participated in the study. The test meals consisted of 250 ml
of water blended with 50g of glucose and GILP powder in escalating doses (0g, 5g, 10 g and 20 g GILP) and was tested in a randomized order at breakfast after an overnight fast. Results show that blood glucose concentrations were significantly reduced at 30, 45, and 60 min after the 20g GILP protein meal when compared to the control. In conclusion, GILP lowers postprandial glycemia when added to 50 g of carbohydrates. The authors suggest that addition of GILP to carbohydrate food may be an effective way to lower the glycemic response of the foods. (Petersen et al. 2009)

Frid et al. (2005) investigate whether supplementation of high GI meals with whey proteins may increase insulin secretion and improve blood glucose control in type 2 diabetic subjects. Fourteen diet treated type 2 diabetic subjects obtained a high-GI breakfast and a subsequent high-GI lunch. The breakfast and lunch meal contained 18.2 g whey one day and another day it was exchanged for lean ham and lactose. Result show that the blood glucose response (AUC) after breakfast was not significantly different after the whey meal compared to the reference meal when evaluating AUC. On the other hand, the AUCs of insulin response corresponding to the whey breakfast were significantly higher than after the reference meal. The results from the subsequent lunch meal show that blood glucose levels were significantly lower when whey was included in the lunch compared to the reference meal. Subsequently, the insulin response was elevated after the whey intake compared to intake of the reference meal. After both breakfast and lunch including whey, GIP levels where significantly higher whereas no significant differences were observed in the GLP-1 response. The authors suggest that GIP responses may be responsible for higher insulin response and subsequent lowering of blood glucose. However, in diabetic patients this mechanism is more uncertain because the incretin system effect appears to be impaired as a consequence of deteriorated secretion of GLP-1 and loss of insulinotropic activity of GIP. Today is sulfonylureas used as a stimulator of insulin secretion and it also attenuates postprandial blood glucose. A disadvantage with this treatment is that it can cause hypoglycaemia. The authors hypothesis that an attractive thought is to use whey proteins as a valuable tool and even an alternative treatment in managing diabetes type 2 where a daily intake of whey could facilitate normoglycemia (Frid et al. 2005)

It could be hypothesised that the insulinogenic property of milk may be attributed to other components of milk products, such as fat for example. According to Hoyt and colleagues (2005) when comparing the glycemic index (GI) and the insulin index (II) of both skimmed and whole milk in nine healthy subjects, no significant differences were observed between GI and II for skimmed and whole milks. Therefore it is concluded that the dissociation of the GI and the II in milk is not related to its fat content. (Hoyt et al. 2005)

3.1.3 Proposed mechanisms from animal studies

A study in mice suggests that the effect of dairy in diabetes type 2 might be explained by the calcium and vitamin D content of milk. The proposed mechanism for this is that pancreatic b-cells contain vitamin D receptors (VDR), 1a-hydroxylase enzyme and calcium binding proteins. These components respond to the active form of vitamin D (1,25(OH)2D3) which results in an increased insulin secretion. The thought that vitamin D may have a pancreatic effect is given by results of studies performed in mice that has not got a functional VDR. Mice lacking a functional VDR show a lower level of circulating insulin and higher plasma glucose compared with mice that has functional VDR (Arajau et al. 2009)
Matsumoto and coworkers (2009) evaluate the effect of long-term daily cows milk administration on insulin resistance induced by a high-sucrose diet on a total of 24 rats. The rats where divided into four groups (6 rats in each group) and were fed with either sucrose or a dextrose diet for seven weeks. Within each diet assignment, one of the two groups was orally administered with 25 ml per kg body weight of either artificial milk or cows milk. Results from this study show that the cow’s milk treated group demonstrate lower fasting insulin and fructosamine levels than a artificial milk-treated group. Improved insulin sensitivity due to cows milk administration seemed to be associated with reduced duodenal GLUT2 mRNA levels and increased propionate production within the caecum. The authors concluded that long-term daily cows milk administration improves insulin resistance induced by a high-sucrose diet and that cows milk is effective in counteracting the metabolic syndrome even at reasonable concentrations. (Matsumoto et al. 2009)
4. Effect of milk and dairy intake on blood pressure

4.1 Epidemiology

4.1.1 Evidence from observational studies

Alonso et al. (2009) analyzed data from The ARIC study, a populations based prospective cohort study that aim to assess whether different types of dairy products were associated with changes in blood pressure over nine years among a white and an African-American population. In total, the analysis included 8208 men and women aged 45-64 years in the US that were followed for nine years. At baseline, participants underwent a physical examination, including measurements of blood pressure and dietary assessment using a food frequency questionnaire. Six items corresponded to dairy products (low-fat milk, whole-fat milk, yogurt, ice cream, ricotta or cottage cheese and other cheese respectively). The ARIC participants were examined 3 times during the follow-up at approximately 3-year intervals.

The results show that only low-fat milk was inversely associated with systolic blood pressure longitudinally, and this association was restricted to whites. Systolic blood pressure between visit 1 and 4 (9 years later) increased 2.7 mmHg less in whites consuming 3 or more daily servings (one serving of milk correspond to 237 ml) of low-fat milk than in those consuming less than 1 serving per week. Neither intake of whole-fat milk in whites, nor any dairy food in Africa-Americans was associated with blood pressure changes over time.

In conclusion, the results from this study offer some evidence that higher low-fat milk intake might prevent blood pressure elevations associated with age, at least in whites. (Alonso et al. 2009)

Engberink and co-workers (2009) aim to examine the association of dairy intake and the incidence of hypertension in 2245 men and women aged ≥55 years in a population-based prospective cohort study with 6-years of follow-up period. The study participants had complete dietary and blood pressure data and were free of hypertension at baseline. The median energy-adjusted total dairy intake of the study population was 396 g/day, ranging from 1 serving/day (164 g/day) in the lowest quartile to 4.5 servings/day (691g/day) in the highest quartile. After 2-year of follow-up 664 subjects had developed hypertension. Results show that each increase in number of dairy serving per day (150 ml) was associated with 7% lower risk of hypertension. When the association was stratified by low-or high-fat dairy product, the significant inverse association was primarily limited to low fat dairy intake. After 6 year of follow-up, 984 subjects had developed hypertension, and the association between dairy intake and hypertension was attenuated, with hazard ratios of 0.81 for both total and low-fat dairy intake in the upper versus lower quartiles of dairy intake. These results raise the hypothesis that dairy product intake may slow the progression rather than prevent the development of hypertension in certain individuals because of the results seen from the 2 year follow-up compared to 6 year of follow-up. It might also be that subjects who are most susceptible to a beneficial effect of dairy products may already benefit after 2 years, whereas individuals who do not respond within 2 years will also not respond after 6 years.

In summary, authors conclude that intake of low-fat dairy products may contribute to the prevention of hypertension at an older age. (Engberink et al. 2009)

Djoussé and colleagues (2006) perform a cross-sectional study where the association between dairy products intake and prevalence of hypertension is investigated.

In total, 4794 participants from the National Heart, Lung, and Blood Pressure institute (NHLBI; phase 1 1993-1995 and phase 2 1994-1995) family heart cohort study composed the
study population. The dairy intake was estimated from a staff-administrated semi-quantitative food frequency questionnaire. The consumption of dairy was divided into quartiles from low intake (0.4 servings/day) to high intake (3.1 servings/day).
Results show that subjects in the highest quartile of dairy consumption had 36% lower prevalence for hypertension compared with those in the lowest quartiles. No association between dairy intake and hypertension was seen among subjects whose saturated fat intake was above median (11.2 E%). However, a stronger inverse association was seen among subjects who consumed <11.2E% saturated fat.
From the lowest to the highest quartiles of calcium intake there was an inverse associations between calcium intake and prevalence of hypertension. Blood pressure was lowered by 2.6 mmHg in systolic blood pressure comparing the highest with the lowest quartile of dairy intake. The association was even stronger in subjects who had an intake of saturated fat below median.
In conclusion, the data from the study suggests that there is an inverse association between dairy intake and prevalence of hypertension that is independent of dietary calcium, mainly among subjects consuming less saturated fat. This might lead to the thought that consumption of low-fat dairy products might be more beneficial for preventing hypertension. (Djoussé et al. 2006)

Jorde and colleagues (2000) investigate in a cross-sectional study the relation between calcium and vitamin D intake, and intake of vitamin D from dairy products on systolic and diastolic blood pressure in adults. In total, 15 596 men and women aged 25-69 years, from Tromsø, Nordern Norway participated in the study during 1994-1995. The participants filled out a food frequency questionnaire at baseline, blood pressure, weight, and height data were collected for all participants.
Results show that in both sexes there was a significant linear decrease in systolic and diastolic blood pressure with increasing dairy calcium intake. However, the difference in blood pressure in subjects with the highest and those with the lowest calcium intake was only ≤ 1.3 mmHg. Vitamin D intake had no significant effect on blood pressure. Small reductions in blood pressure, as seen in the study, might be related to the calcium intake from dairy sources.
In conclusion, there is a negative association between calcium intake from dairy products and blood pressure. This could be of importance in the preventing primarily stage of cardiovascular diseases.
(Jorde et al. 2000)

Beegom et al. (1997) performed a cross sectional study that aim to demonstrate whether saturated fat intake may be a risk factor of hypertension. In total, 1497 South Indian men and women were included in the study and their dietary intake was estimated by a pretested and validated food frequency questionnaire. Height, weight and blood pressure were measured. The mean intake of dairy foods (milk and yogurt) was among men 135 g/day and among women 120 g/day.
The results from this study show that in all subjects, the consumption of milk and yogurt and butter among other nutrients where significantly higher in hypertension compared to nonhypertensive subjects.
In conclusion, the present study show that higher intake of saturated fatty acids, particularly coconut oil, milk, butter, and yogurts and other are risk factors of hypertension in a south Indian population. (Beegom et al. 1997)
4.1.2 Evidence from intervention studies

The Dietary Approaches to Stop Hypertension (DASH) study investigate the effects of dietary patterns on blood pressure as well as combined effects of nutrients that occur together in food in a randomized blinded controlled trial. The study group consisted of 459 slightly hypertensive men and women. For 3 weeks all subjects were assigned a control diet consisting of a typical American diet low in fruits, vegetables, and dairy products, with a fat content of a typical average diet (approximately 37% fat). After this run-in period the subjects were randomly assigned to one of 3 intervention diets; 1) the control diet, 2) a fruit-and-vegetables diet or 3) a combination diet rich in fruit, vegetables, and low-fat dairy foods with a reduced amount of saturated fat, total fat, and cholesterol. The study participants had to follow the assigned diet for 8 weeks. Once each week, staff members who were blinded to the diet assignment measured blood pressure.

Results show that the fruit-and-vegetables diet reduced the systolic blood pressure with 2,8 mmHg and the diastolic blood pressure with 1,1 mmHg compared to the control group. The combination diet, reduced the systolic blood pressure with 5,5 mmHg more and diastolic blood pressure with 3,0 mmHg. Among the subgroup of with hypertension (133 subjects), the combination diet reduced systolic and diastolic blood pressure by 11,4 and 5,5 mmHg, respectively.

In conclusion, the diet rich in fruit, vegetables and low-fat dairy food with reduced saturated fat content (6 E%) and total fat (27 E%) can substantially lower blood pressure in mildly/moderately hypertensive persons. (Appel et al. 1997)

Hilpert et al. (2009) aim to examine the effects of dairy foods on blood pressure and intracellular calcium and the dependence of blood pressure changes on changes in intracellular calcium. In total, 23 men and women with untreated stage 1 hypertension (SBP= 140-150 and/or DBP= 90-99 mmHg) were randomly in a cross-over study design assigned to either an average Western diet (AWD) as control, a diet high in fruits and vegetables (F&V), and a similar F&V diet containing 3,4 servings/day of dairy products (D-F&V) for 5 weeks each. The D-F&V diet included low-fat and non-fat milk and yogurt and full-fat cheese. The AWD diet included full-fat dairy products while the F&V diet included lower fat dairy products.

The results from this study show that systolic and diastolic blood pressure were significantly reduced by ~2 mmHg following both D-F&V and F&V diets vs. the control (AWD). The D-F&V diet lowered intracellular calcium compared to the other two diets, and this change correlated with the fall in DBP.

In conclusion, the findings in this study suggest that blood pressure responses to increasing dietary calcium is associated with attenuation of the widely reported elevation in intracellular calcium in hypertensive subjects. (Hilpert et al. 2009)

Alonso et al. (2009) perform a randomised cross-over trial that aim to investigate whether supplementing diets with low- or whole-fat dairy products would differentially affect blood pressure levels and weight. In total, 48 normotensive men and women were randomly assigned to either drink 3,5 servings (250g serving size) of whole-fat or low-fat dairy products for 8 weeks with a wash-out period of one month in between.

Results show that intake of whole-fat dairy significantly increases systolic blood pressure by 2,1 mmHg and weight by 1,2 kg but not diastolic blood pressure. An increase in intake of low-fat dairy products did not significantly affect systolic or diastolic blood pressure.

In summary, this small cross-over trial did not reveal any comparative benefit of low-fat dairy compared with whole-fat dairy products on blood pressure levels. In addition, the present
results indicate a possible undesirable effect of whole-fat dairy product intake in some individuals, probably linked to elevated levels of saturated fats and high caloric content. (Alonso et al. 2009)

Van Meijl et al. (2009) aim to examine the effects of daily consumption of low-fat dairy products on metabolic risk parameters in a randomized study with a cross-over design. The study group consisted of 35 overweight (BMI >27 kg/m^2) men and women who were randomly assigned to either a control diet or a dairy diet for 8 weeks. The dairy group consumed 500 ml 1.5% fat milk and 150 g 1.5% fat yogurt per day. The subjects in the control group consumed 600 ml fruit juice and 43 g biscuits per day. Blood pressure and metabolic risk parameters (serum total cholesterol, LDL-cholesterol apo B, triacylglycerols, non-esterified fatty acids, glucose, insulin, C-reactive protein and plasmin activator inhibitor-1) were measured.

Results show that systolic blood pressure was significantly decreased by 2.9 ± 7.4 mmHg after dairy consumption. Metabolic risk parameters were unchanged during the intervention period.

In conclusion, authors suggest that a daily consumption of 500 ml 1.5% fat milk and 150g 1.5% fat yogurts for eight weeks reduces systolic blood pressure in overweight and obese subject but do not improve metabolic risk parameters. (Van Meijl et al. 2009)

**Effect of milk peptides on blood pressure**

In a meta-analysis performed by J.Y. Xu et al. (2008) including 12 trials, the effect of milk tripeptides (lactotripeptides) on blood pressure in prehypertensive and hypertensive subjects was summarised.

Results show that the average effect of IPP and VPP on systolic blood pressure was a decrease of 4.8 mmHg for systolic blood pressure and for diastolic blood pressure 2.2 mmHg, this effect being even greater among the hypertensive subjects. In nine of these trials, IPP and VPP were produced from casein by proteolysis by L. helveticus, and in the other three trials by Aspergillus oryzae. The ingested amounts of each peptide in the trials were 2.6-5.6 mg/day.

Similar results were observed in a meta-analysis performed on the effect of peptides derived from food proteins and their affect on blood pressure by Pripp (2008). The effect of milk peptides on systolic blood pressure was −5.13 mmHg and for diastolic blood pressure −2.42 mmHg, where the tested amounts of the peptides ranged from 1.5-30 mg VPP and 1.1-22.5 mg IPP.

In conclusion, these analyses provide evidence that milk derived peptides such as IPP and VPP have a hypotensive effect in prehypertensive and hypertensive subjects. Milk peptides could therefore be a supplement or an alternative to pharmaceutical treatment for mild hypertension. Due to the fact that both fermentation of milk as well as digestion of milk proteins results in a huge amount and variety of different peptides, further randomized control trials are needed to test the hypotensive effect of these milk and fermented milk. (Xu J-Y et al. 2008, Pripp 2008)

In contrast to the above, there are three studies showing no effect of tripeptides on blood pressure. Engbrink and coworkers (2008) perform a double-blinded randomized controlled trial (RCT) that aim to examine whether daily intake of 14 mg lactotripeptides (IPP and VPP), obtained by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis would
influence blood pressure over a 8-week period in 135 subjects with elevated blood pressure, \( \geq 140 \text{ mmHg} \).
The dairy drinks were low-fat yogurt that was ready-to-drink containing IPP and VPP. Daily intake of the drinks per subject was 200 ml, containing 4.2 to 5.4 mg IPP and 5.0 to 5.8 mg VPP.
Results show that there was no significant effect of lactotripeptide treatment on systolic blood pressure. The mean difference between treatment and placebo was 2.8 mmHg for concentrated fermented milk based lactotripeptides, -0.5 mmHg for enzymatic hydrolysed lactotripeptides and 1.6 mmHg for synthetic LTP.
In conclusion, no effect was seen on the systolic and diastolic blood pressure with the LTP treatment. (Engberink et al. 2008)

Van der Zander et al. (2008) perform a multi-center, double-blinded, parallel, placebo controlled trial that aim to investigate the hypothesis that consumption of enzymatically hydrolyzed lactotripeptides (ELTP) in a yogurt beverage lowers blood pressure in a eight-week intervention study. In total, 275 subject (35-70 years) with an average systolic blood pressure between 140-170 mmHg and diastolic blood pressure <100mmHg participated in the study. The participants were randomly assigned to receive either the test or the control product without ELTP. The incorporated ELTP formulation was in a yogurt drink and, over a period of 8 weeks participants had to consume 200 g of the testing product at breakfast. The substance added to the yogurt was an enzymatically hydrolyzed casein powder by Aspergillus oryzae protease. The test product (200 g ELTP-enriched product) contained 28.9 mg IPP/L and 22.0 mg VPP/L.
Results show that 10.2 mg of ELTP/day did not reduce systolic blood pressure or diastolic blood pressure compared with the placebo.
In conclusion, the study showno effect of an ELTP-enriched yogurt beverage on blood pressure in hypertensive subjects in a fairly large study. (Van der Zander et al. 2008)

Lee and coworkers. (2007) aim to investigate the effect of a milk drink supplemented with whey peptides on blood pressure in mildly hypertensive subjects. A total of 54 patients received either 125 ml of a milk drink, supplemented with whey peptides (2.6 g/100 g) every morning or a control products for 12 weeks.
The results from the study show that resting systolic and diastolic blood pressure values did not change in the milk group 144.1 \pm 8.6/ 91.0 \pm 5.5 mmHg at baseline versus 143.7 \pm 13.5/ 90.4 \pm 6.5 mmHg after 12 weeks.
The authors concludes that daily consumption of 125 ml of a milk drink supplemented with whey peptides was not found to reduce blood pressure in mildly hypertensive subjects. A possible explanation for the missing effect could be degradation of the peptides by intestinal or plasma peptidase before they could exert an effect on blood pressure. (Lee et al. 2007)

4.1.3 Proposed mechanisms from animal studies

Other possible mechanism explaining the blood pressure lowering effect of milk has been suggested. Casein peptides have been discovered to have opioid-like activity. Opioids bind to opioid receptors and have morphin-like effects they are present in the central nervous system where they are involved in the regulation of circulation and can also affect blood pressure, although the mechanism is still unknown. The first characterized opioid milk peptide agonist beta-casomorphin was derived from beta-casein. There are milk peptide agonists derived from alfa-casein (alfa-exorphins) and from kappa-casein (casoxins). In spontaneously hypertensive
rats, opioid peptides derived from whey have been shown to lower blood pressure. (Jauhiainen 2007 et al., Czapla et al. 1998)

The role of potassium in the blood pressure lowering mechanism has been studied in animal models. A low potassium intake has been shown to increase the sodium-hydrogen exchange system, which results in an induced acidosis and also in a stimulation of the renin-angiotensin system and sympathetic nervous system. Why this occurs is still unclear but a probable mechanism could be that an increase in sodium-hydrogen activity in vascular smooth muscle cell may elevate cellular sodium concentrations, and reduce calcium efflux through the sodium-calcium exchange. This results in elevated calcium concentrations in the cytosol and lead to vasoconstriction. Another mechanism that also affects vasoconstriction is when low potassium levels are present and the sodium-potassium-ATPase activity is decreased. As a result, intracellular sodium is elevated which results in a slower exchange in the sodium-calcium activity. The excretions of calcium in the urine decrease while intracellular concentrations increases and it affects vasoconstriction.

When extracellular magnesium was increased in experimental animals, it resulted vasorelaxation, decreased vascular resistance, and attenuated agonist-induced vasoconstriction. The opposite effect was seen when concentrations of magnesium where decreased causing contraction, potentiating agonist-induced vasoreactivity, and increased vascular tone and blood pressure. (Kris-Etherton et al. 2009)
5. Effects of milk and dairy intake on central abdominal fat

5.1 Epidemiology

5.1.1 Evidence from observational studies

Rajpathak et al. (2006) conducted a prospective cohort study (12 years of follow-up) of 43 119 men (aged 40-75) from data of the Health Professional Follow-up Study. The study aim was to investigate the association between calcium and dairy intake and 12-years weight change in US men. The baseline analysis was conducted on 23 504 men and the change of intake on 19 615 men. The average frequency of consumption was assessed by a semiquantitative food frequency questionnaire were calcium intake from dairy was calculated as the sum of dietary and all supplement calcium intake. Results show that total calcium intake was not significantly associated with weight change (in a multivariate analysis with adjustment for potential confounders). In addition, no association with dietary, dairy or supplemental calcium intake and weight change was found when evaluation was made separately. The difference in mean weight gain between extreme quintiles of high-fat dairy intake was small, which indicates that the subjects who consumed more high-fat dairy at baseline had a slightly lower 12-years weight gain that the subjects who consumed less high-fat dairy. On the other hand, there was no significant association with weight change and low-fat dairy intake.

The authors conclude that the findings of this study do not support the hypothesis that increasing calcium or dairy consumption is associated with lower long-term weight gain in men. (Rajpathak et al. 2006)

In a prospective cohort study by Vergnaud et al. (2008) the relation between dairy consumption and calcium intake with 6-years change in body weight and waist circumference is investigated. The sample consisted of 2267 men and women, aged >45 years of age. A computerized dietary record estimated total dairy products were used. Three specific dairy products were included, milk, cheese and yogurt, expressed as servings per day. BMI and waist circumference measures were performed on study participants at baseline and at follow-up.

Results show that total dairy consumption, especially milk and yogurt, is inversely associated with 6-years changes in weight and waist circumference in men who were overweight at baseline. These associations were not explained by calcium intakes. In contrast, a positive relation was observed between weight change and yogurt consumption in normal-weight women, whereas a positive relation between milk consumption and waist circumference change was observed in overweight women.

In summary, authors suggests that the negative association between dairy products and anthropometric changes observed in overweight men is not explained by dairy calcium intakes, which gives rise to the thought that other components of dairy products or specific dietary pattern associated with dairy consumption may help to explain the results. (Vergnaud et al. 2008)

Moore and colleagues (2008) aim to investigate the relation between dairy intake and body fat among children and adolescents in a cross-sectional study. The study population was composed of 3864 children and 1884 adolescents in NHANES III (from 1988-1994) and 2231 children and 2636 adolescents in NHANES (from 1999-2002). The dietary intake was in NHANES assessed by means of 24-h recall interviews whereas in NHANES III a
microcomputer-based interview was performed. One serving of dairy was defined as 8 ounces (appr. 200 g) of milk or yogurt, 1.5 ounces (appr. 36 g) of natural cheese or 2 ounces (app. 48 g) of processed cheese. The anthropometrical measurements were BMI and skin-fold thickness (sum of triceps and subscapular) to obtain indices of body fat. For girls, the cutpoints to represent low, moderate or high intake of dairy were <1, 1-3 and servings/day and for boys <2, 2-4 and ≥4 servings per day.

Results show that after adjusting for age, height, race/ethnicity and television watching, there is no evidence that higher dairy intakes are associated with excess body fat (BMI or skinfold measures) for either children or adolescents. In fact, adolescent girls and boys who consumed less dairy had consistently higher BMI and a larger sum of two skinfolds. Further analysis show that adolescents girls consuming 1-<3 servings of dairy per day compared with those consuming < 1 serving /day had less fat in their sum of two skinfolds; girls consuming ≥3 servings per day had even less fat in their sum of two skinfolds. Same results were seen among boys where a higher dairy intake was associated with lower anthropometric measures of body fat.

In summary, authors conclude that among adolescents, suboptimal dairy intake was associated with higher anthropometric measures of body fat. (Moore et al. 2008)

Brooks and co-workers (2006) aim to examine the association between calcium intake and dairy product consumption and overweight and obesity in young adults. A cross-sectional design with data from The Bogalusa heart study (1995-1996) was used. The study participants consisted of 1306 young men and women aged 20-38 years. The intakes of calcium from dairy products were obtain from self-administered semi quantitative food frequency questionnaire where 1 serving per week was converted to 0, 14 serving per day. Milk was classified as whole milk, 2% milk, 1% milk or skim/non fat milk as well as type of yogurt and cheese. BMI, waist-to-hip ratio and average waist circumference measurements were performed for each participant.

In white males results show, a negative association between intake of calcium and low-fat dairy products and overweight only when waist-to-hip ratio was used to define overweight status. Among all study participants, there was no significant association between dairy products consumption, calcium intake and overweight when it was defined by BMI.

From the results seen in the study, it is suggested that intake of calcium and low-fat dairy products is inversely associated with abdominal adiposity, particularly in white males. (Brooks et al. 2006)

Jacqmain et al. (2003) studied the association between daily calcium intake and body composition and plasma lipoprotein-lipid concentrations in a cross-sectional study based on data from the Québéc family study. The study sample consisted of 235 men and 235 women (20-65 years). A 3-day food record assessed daily intakes of nutrients, including milk and dairy products (milk, cheese, yogurt, ice cream, pudding and desserts with milk and soups prepared with milk).

Results from the study show that women who consumed <600 mg dietary calcium per day had higher values of body weight, BMI, percentage of body fat, fat mass, waist circumference and abdominal adipose tissue than those who consumed ≥600 mg per day. No significant differences were found across subgroups of men. Most of the dietary calcium came from dairy products (62% for women and in 60% for men).

The authors conclude from these results that a low-calcium intake is associated with greater adiposity, particularly in women who report a low calcium intake. (Jacqmain et al. 2003)
A total of 19,352 normal-weight women were included in a prospective cohort study performed by Rosell and colleagues (2006), which aim to examine the association between changes in dairy product consumption and weight change over 9 years in middle-aged Swedish women. Data on the study sample was taken from the Swedish mammography cohort study. Dietary intake was measured by a 67-item food frequency questionnaire. Dairy product consumption included whole milk/sour milk (3% fat), medium-fat milk (1.5% fat), low-fat milk and sour milk (≤0.5% fat), cheese and butter (80%). The subjects were categorized into 4 groups according to their change in intake (increased or decreased intake) of these foods during the 9 years of follow-up.

Results show that for women with a low intake of whole milk and sour milk, cheese, and butter, BMI was significantly higher than in women with a high intake of these products. For women with a low intake of low-fat milk and sour milk, BMI was significantly lower in subjects with a low intake. Normal-weight women with a constant intake of ≥1 serving per day of whole milk and sour milk, and cheese during the follow-up time, had a significantly lower risk of gaining ≥1 kg/year than did women with a constantly lower intake of these products.

In conclusion, the association observed between the intake of dairy products and weight gain differed according to the type of dairy product and according to the body weight status of the women at baseline. (Rosell et al. 2006)

5.1.2 Evidence from intervention studies

Wennersberg and colleagues (2009) aim to test the hypothesis that an increased proportion of dairy products in the diet might beneficially affect abdominal obesity, and/or other aspects of the metabolic syndrome. The study group of this 6-month randomized, parallel-group intervention study was composed of 121 middle-aged overweight men and women (BMI >38) that where low-consumers of dairy products. The study participants were instructed to include 3-5 portions of milk/yogurt and other dairy products in their daily diet (cheese, creme fraiche, butter, cottage cheese). Subjects in the control group maintained their habitual diet. Clinical and laboratory test were conducted at baseline and at the end of the 6-month intervention.

Results from the study show that body weight, BMI, waist circumference, and sagittal abdominal diameter remain unchanged during the intervention, as did body fat mass and the proportion of body fat. Results from a subgroup of the material, the effects related to habitual calcium intake show that in subjects with a low calcium intake at baseline, waist circumference was 102.1 ± 10.2 cm compared to 99.3 ± 10.8 cm after the 6 month intervention period in the milk group. However, no change in waist circumference was observed in the control group.

The authors conclude that the study does not give a clear support to the hypothesis that a moderately increased intake of dairy products beneficially affects aspects of the metabolic syndrome. The apparently positive effects of additional calcium on waist circumference and sagittal abdominal diameter in subjects with a concomitant low calcium intake, suggest a possible threshold in relation to effects on body composition. (Wennersberg M et al. 2009)

Zemel et al. (2004) performed a randomized placebo-controlled trial in 32 obese men and women (BMI 30-39 kg/m²) with the objective to determine the effects of increasing dietary calcium on body weight and fat loss, secondary to an energy-restricted diet with an energy deficit of 500kcal/day. Individuals were assigned to a 500kcal/day deficit diet either as 1) a control diet, including 0-1 serving of dairy products/day (type of dairy not defined), 400-500
mg Ca/day and a daily placebo supplement; 2) a calcium supplemented diet identical to the control diet, with the placebo replaced by 800 mg Ca/day of calcium to give a total of 1200-1300 mg Ca/day; 3) a high-dairy diet (placebo supplemented) and containing three daily servings of dairy products, to give a total of 1200-1300 mg/day of dietary calcium. The diet was assigned for 24 weeks.

Results show that the study participants on the low-calcium control diet lost approximately 8 kg of their body fat. The subjects who consumed the high-calcium diet lost 11.6 kg and the group on the high-dairy diet lost 14 kg. Results were significantly different between the groups.

In summary, an increase in dietary calcium to an energy restricted diet affected weight and fat loss positively as well as percentage fat loss from the trunk. (Zemel et al. 2004)

Lorenzen et al. (2007) aim to examine the acute effect of a high calcium intake from dairy products, or from supplements on postprandial fat metabolism and appetite in a randomized cross-over study. Four different isocaloric meals were tested on 18 moderately overweight subject (BMI 24-31 kg/m²). The study participants were randomly assigned to one of the test meals and each test meal day lasted 8.5h with a wash-out period of three weeks between the test days. The test meals contained dairy proteins as the main protein source, and 1) a high amount of calcium from dairy products (793 mg Ca; HC meal), 2) a medium amount of calcium from dairy products (350 mg Ca; MC meal), 3) a low amount of calcium from dairy products (68mg Ca; LC meals), 4) or a calcium supplement served as a drink containing calcium carbonate (850mg Ca; Suppl meal). Body composition was measured at baseline and at the end of the intervention with the bioelectric impedance method, and fat-free and fat mass were calculated. Visual analogue scales was used to measure subjective appetite sensation/rating.

Results show that a high calcium intake from dairy products, milk and low-fat yogurt, but not from a calcium supplement, decrease postprandial lipidemia. Compared with the LC meal, the lipid response in chylomicron triacylglycerol (expressed as area under the curve for chylomicron triacylglycerol) was reduced by 17% by the MC meal and by 19% by the HC meal, which indicate that a high calcium intake from dairy products reduces the lipid response but also that a plateau value exists above which an increased calcium intake dose not seem to have any additional effect. No consistence effects of calcium on appetite sensation was observed.

In summary, authors conclude that an increase in calcium intakes from dairy products attenuate postprandial lipidemia, most probably because of reduced fat absorption, whereas supplementary calcium carbonate does not exert such an effect. (Lorenzen et al. 2007)

Bendesen et al. (2008) aim to investigate the effect of calcium from dairy sources (yogurt, milk, cream and butter) on fecal fat excretion in a randomized cross-over trial including 11 men and women with BMI 25-31 kg/m². Two isocaloric diets were randomly assigned between subjects; 1) a diet containing 205 mg of calcium (high-Ca) from low-fat dairy products and 2) a diet contained 52 mg of calcium (low-Ca) from low-fat dairy products. Two 7-day dietary intervention periods was undertaken with had a wash-out period of 1 week between.

Results show that after the high-Ca diet, the fecal fat excretion increased significantly (more than twofold) compared with the low-Ca diet (11.5 g/d fecal fat vs. 5.4 g/d fecal fat). In contrary to the above, total fecal excretion of bile acids did not differ in the two diet. One possible explanation to this is that an increase in dietary calcium would result in increased lipolysis and decreased lipogenesis resulting in a stimulation of fat loss. Another possibility is that calcium interferes with fat absorption in the intestine by forming insoluble calcium soaps.
with fatty acids or by forming precipitates with phosphate and bile acids, resulting in decreases in the digestible energy of the diet. Authors concludes that the results may partially explain why a high-calcium diet can produce weight loss. (Bendsen et al. 2008)

Bortolotti and colleagues (2008) perform a double-blinded, placebo controlled, randomized crossover study including ten (3 men and 7 women) overweight subjects (BMI>27kg/m²). The aim of the study was to investigate the effect of dairy calcium supplementation on markers of fat metabolism. All of the subjects had low daily intake of calcium (<800 mg/d) and were randomly assigned to receive either a placebo or a calcium supplement (800 mg/day) for a period of 5 weeks. The calcium supplement was a natural milk mineral concentrate. The effect of calcium supplementation on fasting whole-body lipid turnover and lipid disposal was measured as well as in vivo lipid metabolism under normal and lipolysis-stimulated conditions in subcutaneous adipose tissue. Results from this study show no significant difference between body weight between the groups at the end of the intervention. There was no significant effect on lipid metabolism when biomarkers of fat metabolism of subcutaneous adipose tissue were examined. In conclusion, the study show that dairy calcium supplementation in overweight subjects with a low calcium intake did not affect fat metabolism and energy expenditure under resting conditions. (Bortolotti et al. 2008)

The effect of milk and dairy on satiety and satiation

Milk is high in protein and might be expected to exert a greater satiating effect than drinks composed predominantly of carbohydrates. This could be useful if it can reduce the subsequent energy intake at a meal and therefore contribute to weight loss. Tryptophan (TRP), an amino acid present in whey, may contribute to the satiating effect, as it serves as a precursor for the anorexigenic neurotransmitter serotonin. Nieuwenhuizen et al. (2008)

Dove and colleagues (2009) aim to investigate the effects of skim milk in comparison with a fruit drink on self-reported postmeal satiety and energy intake at a subsequent meal in a randomized controlled crossover designed trial. The study group was composed of 34 overweight men and women aged 25-70 years. Subjects consumed 600 ml of skim milk or a 600 ml fruit drink with a fixed-energy breakfast, an ad libitum lunch was provided 4 h after breakfast. Height and weight to calculate BMI as well as systolic and diastolic blood pressure were measured. Participants completed visual analogue scale ratings of their satiety before breakfast, throughout the morning, and immediately after lunch. The results from the study show that energy intake at lunch was significantly lower after consuming skim milk than after consuming a fruit drink. This result was supported by self-reports of fullness, satisfaction, and prospective consumption that indicated significantly lower perceived appetite after consumption of skim milk than after consumption of a fruit drink.

The study results suggest that consumption of skim milk instead of a fruit drink will lead to increased satiety and decreased energy intake at the next meal. (Dove et al. 2009)

Chung Chun Lam et al. (2009) aim to determine the short-term effect of a mixtures of whey protein and glucomacropeptide (GMP) versus a carbohydrate control, on satiety. Fifty healthy men and women were recruited in a randomized single-blinded, cross over designed study. Study participants received a subject-specific breakfast, a preload drink and lunch. The
preload drink was composed of either 1) maltodextrin carbohydrate (control), 2) whey protein isolate (WPI) with no GMP, 3) WPI with naturally present 21% GMP or 4) WPI with naturally present 21% GMP plus added GMP. Satiety was assessed using visual analogue scales (VAS) and by determining ad libitum food intake at lunch. Results show that subjects rated feeling more full with the lower GMP preload (21% GMP WPI) compared to the carbohydrate control and the other two proteinaceous preload drinks before the lunch test meal. In summary, result suggests that whey with GMP given as a preload on between breakfast and lunch had an effect on the feeling of fullness but this did not translate into a lower intake at the subsequent ad libitum lunch meal. Further work in required understanding dose of protein, delivery mode of preload and timing between preload and subsequent test meal. (Chung Chun et al. 2009)

Veldhorst et al. (2009) aim to compare the effects of casein-, soy-, whey- whey without glycomacropeptide (GMP)-, pure alpha-lactalbumin-, gelatin- or gelatin with tryptophan (TRP) given as breakfasts on subsequent satiety and energy intake at lunch, which was offered 3 h after breakfast. Twenty-four men and women with BMI 24.8 ±0.5 kg/m² participated in the study of randomized, single-blinded design. The breakfast was served as a custard, with either casein, soy, whey, whey-GMP, alpha-lactalbumin, gelatin, or gelatin+TRP.

The results from the study show that alpha-lactalbumin, gelatin or gelatin+TRP containing breakfasts caused a ~20% lower energy intake at the subsequent lunch than did casein, soy or whey-GMP breakfast. The reduced energy intake of 20% was related to a ~40% reduction in appetite.

In conclusion, alpha-lactalbumin, gelatin, and gelatin+TRP given at breakfast are 30-50% more satiating than casein, soy, whey, and whey-GMP. In addition, alpha-lactalbumin, gelatin, and gelatin+TRP induce a 17-24% related reduction of subsequent energy intake at the following meal. (Veldhorst et al. 2009)

Nieuwenhuizen et al. (2008) aim to investigate the role of TRP in the satiating properties of dietary protein. The study group compared three different breakfasts, containing either alpha-lactalbumin (high in TRP), gelatin (low in TRP) or gelatin with added TRP (gelatin+TRP, high in TRP) on appetite in a randomized, single-blinded, cross-over designed study. Twenty-four normal weight men and women aged 19-37 years participated in the study. The breakfast meals were served as a custard and had a protein, carbohydrate and fat content of 10, 55, 35% energy. Hunger ratings were obtained using a visual analogue scale and blood samples were taken on all participants.

The results from the study show that hunger scores were significantly lower in the alpha-lactalbumin breakfast group compared with the gelatin breakfast groups, either with or without added TRP. For each individual amino acid, peak plasma concentrations of asparagines, tyrosine, isoleucine, phenylalanine, leucine, and lysine were higher and glycine, arginine,and ornithine were lower after the alpha-lactalbumin breakfast than after the gelatin and the gelatin with added TRP breakfasts.

In summary, the study show that a breakfast containing alpha-lactalbumin as the only protein source results in a more prolonged suppression of hunger than a breakfast containing gelatin. (Nieuwenhuizen et al. 2008)
5.1.3 Proposed mechanism from animal studies

Zemel et al. (2000) investigate if an increased dietary calcium intake suppress calcitriphic hormones and thereby reduced adipocyte intracellular calcium and lipid storage. To test this theory transgenic mice expressing the agouti gene (obesity gene expressed in human adipocytes) specifically in adipocytes were placed on a low (0.4%) calcium/high fat/high sucrose diet either unsupplemented or with 25-50% of the protein replaced by non-fat dry milk or supplemented to 1.2% calcium supplemented (CaCO₃) for 6 weeks. Results from this study show that weight gain and fat pad mass were reduced by 26-39% by the three high calcium diets. The high calcium diet exerted a corresponding 51% inhibition of adipocyte fatty acid synthase expression and activity and stimulation of lipolysis by 3.4 to 5.2-fold. The authors conclude that an increase in dietary calcium suppresses adipocyte intracellular calcium and thereby modulates energy metabolism and attenuates obesity risk. (Zemel et al. 2000)

De Angel et al. (2009) recently performed a study where they compare the effects of supplemental calcium and dairy calcium on body weight and body composition, glucose and insulin metabolism, and serum adipokines in a mouse model of postmenopausal obesity. Rats (n=108) were randomly assigned to consume a high-fat diet or a low-fat diet containing either; 1) calcium phosphate from non-fat dried milk and whey mineral concentrate (dairy) or 2) calcium carbonate (supplement). The results from the study show that dairy calcium, but not supplemented calcium, decreased weight gain and percent body fat in high-fat fed mice, with no effect on food consumption. Dairy improved insulin resistance and glucose tolerance. Authors conclude from these results that beneficial effects of dietary calcium on body weight and bone health after menopause may be significantly influenced by other dietary components. (De Angel et al. 2009)

Pilvi and colleagues (2009) aim to compare the effect of four different whey protein high-calcium diets (1.8% CaCO₃); 1) alpha-lactalbumin (ALA) 2) beta-lactoglobulin (BLG) 3) lactoferrin (LF) and 3) whey protein isolate (WPI) on weight loss and weight regain in a model of diet-induced obesity. Fecal fat excretion was measured. The results from the study suggest that ALA-meal has beneficial effects on body fat during both weight loss and regain. BLG-meal had a significant effect on body weight but not on the amount of fat tissue. LF-meal accelerated both weight loss and weight regain. In addition to these protein components, whey contains a substantial amount of other minor proteins and peptides, which may have and effect on body weight and can also explain the anti-obesity effect of dairy products. In conclusion, ALA was the most beneficial since it accelerated fat loss during weight loss and the amount of visceral fat was reduced after the weight – regain period. (Pilvi et al. 2009)

A study performed by Boutegourd et al. (2002) compare the effect of three different meals enriched with 1) glucose 2) whole milk proteins or 3) alpha-lactalbumin on the rates of oxidation of glucose, lipids and proteins and on the long-term consequences on body weight and body composition in rats. These parameters were measured in male rats exercised 2 h daily over 5 weeks, either in the fasting state of 1 h after ingestion of a the test meals. The result from the study show that compared with fasting, the whole milk protein meal and the alpha-lactalbumin meals preserved lipid oxidation and increase protein oxidation, alpha-lactalbumin meal increase protein oxidation more than the whole milk protein meal. At the
end of the study, body weight was larger in the whole milk protein-, glucose- and alpha-lactalbumin fed rats than in the fasted ones. This resulted from an increased fat mass in the whole milk protein and glucose rats and to an increasing lean body mass, particularly muscles, in the alpha-lactalbumin rats.

Authors conclude that the potential of alpha-lactalbumin to preserve lipid oxidation and to rapidly deliver amino acids for use during exercise improved the efficiency of exercise training to decrease adiposity. (Bouthegourd et al. 2002)

Suhara and colleagues (2009) examine whether milk exerts peroxysome proliferators-activated receptor (PPAR) agonistic activity in an animal model. The PPAR family consists of three subtypes PPAR\(\alpha\), PPAR\(\delta\) and PPAR\(\gamma\) and belongs to the nuclear receptor family. The results from this report elucidated for the first time that cow’s milk and related dairy products increase the activities of PPAR\(\alpha\), PPAR\(\delta\) and their ligand RXR\(\alpha\). Functional roles of PPAR\(\alpha\) include 1) the regulation of energy homeostasis by activating the fatty acid catabolism, and stimulation of gluconeogenesis and ketone body synthesis 2) the attenuation of inflammatory responses. The activation of fatty acid catabolism induces a decrease in circulating triglyceride levels, a reduction in liver and muscle steatosis and adiposity, which consequently ameliorates insulin resistance. Meanwhile, functional roles of PPAR\(\delta\) include 1) the regulation of energy homeostasis by activating fatty acid catabolism and adaptive thermogenesis, 2) the retardation of weight increase and 3) the control of cell proliferation, differentiation and survival. Thus, PPAR subtypes have become important drug targets for intervention in disease such as hyperlipidemia, diabetes, atherosclerosis, obesity, cancer and Alzheimer’s disease. (Suhara et al. 2009)

Bruckbauer et al. (2009) investigated the effect of additional components in dairy products, branched-chain amino acids and angiotensin-converting enzyme inhibitors, on adiposity and muscle metabolism in a animal model of diet-induced obesity. Mice were for 6 weeks fed with four energy restricted (70%) diets; 1) basal-restricted diet (0,4%Ca), 2) nonfat dry milk (1,2%), 3) calcium-depleted milk (0,4%), 4) or basal-restricted diet with supplemented branched-chain amino acids/ACE-inhibitors. To compare the effects on energy metabolism a high-density oligonucleotide microarray was used.

The results from this study show that lipogenic genes in adipose tissue were downregulated in the milk group while in muscle protein synthetic pathways were stimulated by the Ca-depleted and low Ca/branched-chain amino acid/ACE diet.

The authors conclude that calcium and branched-chained amino acids contribute to the alteration of energy partitioning between adipose tissue and muscle. (Bruckbauer et al. 2009)