ABSTRACT
The demand of healthier products is increasing, and more people are more interested of what they eat. Statistics show that the consumption of snacks is rising.

Hyperglycemia leads to an increased risk for complications in type II diabetes mellitus. Increased levels of postprandial plasma glucose may also lead to equal or maybe more harmful effects than fasting hyperglycemia. When the levels of postprandial plasma glucose are decreased, the development of cardiovascular complications is delayed, why it is important to lower the snacks consumption especially snacks that brings hunger quickly after they are eaten. Because of these factors, healthier products were developed in this study. The aim was to develop a wafer chocolate product that gives higher satiating effect and healthier blood glucose levels compared to one of Cloetta’s chocolate products. Two raw materials were used, a new carbohydrate and a new fat. The new carbohydrate is a healthier sugar alternative than sucrose, since it leads to lower and prolonged increase in blood glucose and insulin levels. The new fat is based on natural oil that is believed to be healthy, mainly due to its satiating effect. The effects of these two materials on blood glucose response and satiety were examined in two products. Furthermore, the products were made of fat reduced milk chocolate in which sucrose in the chocolate mass was 100 % replaced with the new carbohydrate, dietary fibre and fruit concentrate. Only one of the products contained the new fat. The products, together with Cloetta’s chocolate product were consumed by 17 healthy subjects. Blood glucose response and satiating effect after product intake were examined during a period of 3 days.

When blood glucose response was analyzed, a slight indication that the products were relatively healthier than placebo, due to placebo’s unhealthy fluctuations, was found. No clear differences regarding blood sugar maxima were found. Placebo showed, as expected, the highest blood glucose maxima and the largest incremental area under curve, but the maxima of the new fat-lacking product was less than half as high as that of the new fat-containing product and the area was smaller too, which was not expected. The results regarding the hunger levels were not as expected either since the new fat-lacking product was most satiating while the new fat-containing product was the least satiating. Despite that, 57 % of the subjects reported they would by such products in the future.

Several biases may have played a role in the results, for example whether or not subjects followed the criteria (e.g. lunch time, exercise), stress, worry, individual energy requirement and how serious and focused the subjects were. However, for further research, increasing the new fat content to 3 g, a bigger sized product, different filling, more subjects and more repeats of same measurements is recommended.
SVENSK SAMMANFATTNING


När blodglukosrespons analyserades hittades en svag indikation på att produktarna var relativt hälsosammare än placebo, på grund av de ohälsosamma fluktuationerna. Inga klara skillnader med avseende på blodsockermaxima hittades. Placebo visade, som väntat, det högsta blodglukosmaximum och den största arean under kurvan, men maximum för produkten utan det nya fetten av mindre än hälften så högt som det för produkten med det nya fetten och även arean under kurvan var mindre, vilket inte var förväntat. De upplevda hungernivåerna var inte heller som förväntat då Produkten som saknar det nya fetten mättade flest personer medan produkten innehållande nya fetten mättade minst antal personer. Trots det så kunde 57% av deltagarna tänka sig köpa sådana produkter i framtiden.

Flera faktorer kan ha påverkat resultatet, till exempel huruvida försökspersonerna följde kriterierna (t. ex. lunchtid, träning), stress, oro, individuella energibehov samt hur allvarliga och fokuserade personerna var när de angav hungernivåerna. För vidare studier rekommenderas ett högre innehåll av det nya fetten (3 g), en större produkt, annorlunda fruktbasrerad fyllning och fler deltagare och flera upprepningar av samma mätningar.

Keywords: Diabetes mellitus; Postprandial; Hyperglycemia; Fasting; Insulin resistance; HDL; LDL; CHD, CVD; Atherosclerosis; ROS; Flavonoids; Metabolic syndrome; CCK; GLP-1
PREFACE

This project included 20 weeks work, as a part of the Master Degree Programme, Nutrition and Food Chemistry at the University of Kalmar. The planning was carried out at Cloetta Fazer office in Stockholm, the development of the new snacks at the factory Cloetta Fazer in Ljungsbro, and the testing at Gymastik- och Idrotthögskolan in Stockholm.

The project was financed by Cloetta Fazer AB. Blood sugar was measured by two nurses.

Stockholm, July 2008
Dayana Oudah
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1. INTRODUCTION

1.1. Background

More Swedish people are becoming interested in what they eat, showing results from interviews between 1997 and 2006 conducted by the market research company AC Nielsen (1). More people think that the most important thing for the health is to lower the consumption of sugar, and less than half of the Swedish population knows that fat gives more than twice as much calories than sugar. Instead, people think that sugar gives more calories than fat. Often it is overweight people who get bad conscience after sugar consumption, but this is also common among underweight and normal weight people. Candy and soft drinks seem to be the most consumed sugar containing products by Swedes. Chocolate however seems to be more rarely consumed (1).

Statistics compiled by The National Food Administration of Sweden also show that the consumption of snacks, candy and soft drinks rose under the 90th (2). On the other hand, people eat less granulated and cube sugar. The statistics about the sugar consumption are based on the amount that is produced and sold in the country or on diet reviews. However, the sugar consumption varies with age and is higher among teenagers and young adults than among people in the middle age and elderly (2).

Many studies show that hyperglycemia leads to an increased risk for both macrovascular (cardiovascular) and microvascular complications in type II diabetes mellitus. Other epidemiological and experimental studies have shown that increased levels of postprandial (after meal) plasma glucose may also lead to equal or maybe more harmful effects than lasting hyperglycemia. When the levels of postprandial plasma glucose are decreased, the development of cardiovascular complications is delayed. Postprandial hyperglycemia is recognized to be a consequence of insulin resistance and impairment of early insulin secretion by oral glucose load (3). Hyperglycemia is abnormally high blood glucose levels (4).

It is important to lower the snacks consumption, especially those who brings hunger (hypoglycaemic undershoot) quickly after they are eaten, to keep the body weight in control. Based on the earlier mentioned factors and on the increasing interest in more healthy products, we decided to develop a new, healthier chocolate product. The possibility to make a wafer chocolate product that is satiating for a relatively long time and healthier for the blood glucose response was examined. This makes the product differ from the rest of the products that the chocolate and candy industry Cloetta Fazer produce so far. Two different products were made. One of them contains a new fat based on natural oil, and the other does not. The sugar (sucrose) in both products is completely replaced by a new carbohydrate. According to researches, the new fat is supposed to give a long satiating effect that lead to reduced snacking.

Based on the nutrition recommendations of The National Food Administration of Sweden (SLV), the daily energy recommendations, a complete meal should consist of 25-35 % fat (ca 30 %), 10-20 % (ca 15 %) protein and 50-60 % (55 %) carbohydrates. Assuming that two snacks a day are eaten, the approximate daily energy intake of one snack was calculated to 69-411 Kcal, since snacks should provide 5-30 % of the total daily energy requirement (2). The calculations are shown in table I-III.
Table I
SLV recommendations for women versus men

<table>
<thead>
<tr>
<th>Group</th>
<th>Required energy value (Kcal/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>Active 2548</td>
</tr>
<tr>
<td></td>
<td>Inactive 2238</td>
</tr>
<tr>
<td>Men</td>
<td>Active 3285</td>
</tr>
<tr>
<td></td>
<td>Inactive 2905</td>
</tr>
<tr>
<td>Average</td>
<td>2744</td>
</tr>
</tbody>
</table>

Table II
Suggested energy percentage share of total daily energy intake (SLV)

<table>
<thead>
<tr>
<th>Meal</th>
<th>Energy from meal/total energy per day (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>20 – 25</td>
</tr>
<tr>
<td>Lunch</td>
<td>25 – 35</td>
</tr>
<tr>
<td>Dinner</td>
<td>25 – 35</td>
</tr>
<tr>
<td>Between meal snacks</td>
<td>5 – 30</td>
</tr>
</tbody>
</table>

Table III
The required daily energy share of carbohydrates, proteins and fats (SLV).

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>Required energy of total daily energy intake (%)</th>
<th>Population goal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>50-60</td>
<td>55</td>
</tr>
<tr>
<td>Protein</td>
<td>10-20</td>
<td>15</td>
</tr>
<tr>
<td>Fat</td>
<td>25-35</td>
<td>30</td>
</tr>
</tbody>
</table>
1.2. Aim

The aim of this study was to develop a wafer chocolate product as a between meal snack with higher satiating effect and that leads to healthier blood sugar response after oral administration compared to one of Cloetta’s chocolate products.

Healthy blood sugar response is a low, slow and prolonged increase in blood sugar levels without fluctuations. The hunger feeling can be delayed by using a new fat which has been shown to control the appetite. For this purpose, two wafer chocolate products were made and compared to Cloetta’s chocolate product in aim to choose the healthier product in the end. One of the two wafer chocolate products contained the new fat, and the other lacked it. The new fat is based on natural oil that should give higher satiating effect and give better control of the blood glucose response since it, according to scientific studies, increases the release of the hormone GLP-1 which in turn increases the insulin secretion. In both wafer chocolate products a new carbohydrate was used instead of sucrose, which should give healthier blood sugar response compared to Cloetta’s chocolate product. The wafer chocolate products were compared with each other and with Cloetta’s chocolate product as placebo.

The products were consumed by 17 volunteers, their blood sugar levels after intake were measured and their satiating feeling was noted to see whether or not the goal was reached.

The taste of the products was not a key issue in this project due to lack of time and the focus was directed to the effects of the products on the blood glucose and satiating feeling. Despite that, an acceptable taste was reached. Furthermore, a part of the aim was that the products would be fat reduced and have more positive properties like containing antioxidants and dietary fibre. Dietary fibre also leads to a slower increase in blood sugar levels. To obtain a fat reduced chocolate product, a new protein material aimed at such applications was used. Antioxidants were obtained by using a fruit concentrate filling.

From the earlier mentioned description, the following questions were to be answered in this project:

- Are the products healthier than Cloetta’s chocolate product as measured by blood glucose response? How do the blood glucose responses look like for the products in regard to fluctuations, blood glucose maxima, incremental area under the mean curve and time at which the blood glucose levels return to baseline?

- How satiating are the products? Is the product that contains the new fat more satiating compared to the product that lacks the fat and Cloetta’s chocolate product as suggested? Is Cloetta’s chocolate product the least satiating as suggested?

- Does the new protein material work as a fat reducer in these products?

- How does the future market look like for such products?

- What suggestions are there for a different taste, according to the volunteers?

- What else should be thought of in further researches and what should be changed?
1.3. Chocolate

Cocoa is the seed of the cocoa tree, *Theobroma Cacao*, which means “food of the gods” in Greek. The cocoa tree is thought to be cultivated by the original inhabitants of Central America and Northern South America over 3,000 years ago. The cocoa beans undergo fermentation by bacterial oxidative processes. The product of the fermentation is then roasted and grounded into powder (5).

1.3.1. Characteristics of chocolate

Since a long time ago, chocolate has been used as medicine, and today it has been proposed for preventing chronic diseases. Short term randomized feeding trials have found indications that chocolate and cocoa have beneficial effects on cardiovascular disease (6). The chocolate antioxidants act preventive against cancer and cardiovascular diseases as shown by *in vitro* and animal experiments and also in epidemiological studies (7). There are also findings that support the benefits of chocolate flavanols on blood pressure, insulin sensitivity and vasorelaxation (8). Besides, cocoa antioxidants such as epicatechin and procyanidins are shown to be effective *in vitro* in protecting the cells against peroxynitrite, which is a high proinflammatory mediator (7). Chocolate has high content of procyanidin flavonoids, which is one more quality that makes chocolate more beneficial than procyanidin-rich apples (6).

A collection of human studies, observational and randomized trials of either cross-over or parallel design, described the benefits of chocolate (6). Cocoa butter, which is a fat found in cocoa plants and mostly in dark chocolate, consists of stearic acid (33%), oleic acid (33%) and palmitic acid (25%). Palmitic acid is 16:0 saturated; oleic acid is cis- 18:1 mono-unsaturated and stearic acid is 18:0 saturated long-chain fatty acid mainly found in dairy products and meats. Generally, saturated fat is associated to atherosclerosis, thus the risk for cardiovascular disease (CVD). Stearic acid though, might be a non-atherogenic saturated dietary fatty acid. Saturated fatty acids are also often associated with increased total cholesterol and low density lipoprotein (LDL) levels, but a number of studies suggest that stearic acid may not affect the cholesterol, LDL or HDL (high density lipoprotein) levels. A recent trial shows that the effects of stearic acid on lipids are similar to linoleic and oleic acids. The suggested way for how stearic acid acts cholesterol-neutral is the acid’s lower absorption. The absorption rate may be affected by the relative position of stearate on the triglyceride molecule. This might be an explanation to the suggestion that stearic acid from plants, for example cocoa plants, may be different from that from animal derived sources (6).

Compared to corn oil, cocoa butter has lower absorption, show some human feeding trials (6). The heterogeneity has been attributed to calcium’s dual-presence in chocolate. When cocoa butter was supplemented with one weight percent calcium, the butter absorption decreased 13 %. There are two other suggested mechanisms for potential benefit, one is stearic acid’s potential anti-platelet action by reduction of the mean platelet volume, and the other is blood pressure reduction action. Feeding trials found a relationship between stearic acid and coagulation factor VIIc, which is a predictor of fatal CHD (6). However, an increase in the coagulation factor was observed, while in two other trials no change was found (6). There are also additional trials that showed a reduction of VIIc levels by stearic acid, compared to palmitic acid (among others). Regarding stearic acid and blood pressure, some feeding trials
found no negative effect caused by the acid on systolic blood pressure. Other cross-sectional analysis found that the levels of stearic acid could be inversely associated with diastolic blood pressure (6).

In summary, most studies show either beneficial or neutral effects of stearic acid on blood pressure and clotting mechanisms. This includes the factor that stearic acid does not increase CVD risk. Observational studies on the other hand do not give any relationship between stearic acid and CVD. In addition, one case-control study of serum levels found no association with stenosis. There are also contradictions regarding stearic acid and the risk of myocardial infarction (MI) between different observational studies. However, it is important to know that there are several limitations for observational studies due to the high correlations of stearic acid intake with other fatty acids, the minimal contribution of stearic acid in chocolate comparing to the higher content in other food sources like red meats and due to the high interconversion of stearic acid to unsaturated fatty acids. On the other hand, strong evidence from short term randomized trials found that there might a relationship between stearic acid components in chocolate and cardiovascular health (6). In addition, stearic glycerides that are less well absorbed than other glycerides are excreted in the feces. For this reason, cocoa butter has a relatively less bioavailability and has a very little effect on serum cholesterol (5).

Stephan Rössner, a professor at Karolinska Institute and head for the overweight unit, suggests that chocolate is a good copper source and that milk chocolate can be a replacement for apple and sandwich and is a good fibre source as well. Stephan also illuminates that an amount of 40 gram antioxidants in dark chocolate is equal to the amount of antioxidants in a glass of red wine. Milk chocolate can even increase the level of HDL and to decrease the level of triglycerides. The flavonoids increase the breakdown of LDL and prevent fat from going rancid (9). There are evidence that shows a relationship between the oxidation of LDL and the development of atherosclerosis, and inhibition of LDL oxidation may be preventive against atherosclerotic lesions. A Dutch study shows that cocoa polyphenols increase the LDL resistance of LDL to oxidation, and thus suppress the formation of atherosclerosis in Hypercholesterolemic rabbits (10).

1.3.2. Flavonoids in chocolate

Flavonoids are antioxidants from plants and belong to a class called polyphenols with the basic backbone structure C6-C3-C6 of two aromatic rings and different degrees of hydroxylation that distinguish one flavonoid from another. Flavonoids also are divided into different subclasses like flavones, flavanones, flavonols, isoflavones, anthocyanidins and catechins (figure 1). It is shown that cocoa contains high levels of polyphenols, and it is also rich in flavonoids like catechins, epicatechins and procyanidins (polymers of catechins and epicatechins). The flavonoid level in cocoa is greater than in red wine, green and black tea, garlic, strawberry, blueberry, cranberry and apples (6, 11). The flavonoid content in cocoa has been determined to 224 ± 66 µmol/g, in dark chocolate to 124 ± 7.4 µmol/g and in milk chocolate to 52.2 ± 2.04 µmol/g (5).

There are several factors that influence the content of the available phytochemicals in cocoa at the harvest time. An important stage is the proharvest process, due to the fermentation steps most cocoa undergo in heat and acidic conditions. The following steps in the cocoa processing like roasting and alkali treatment lead to a reduction in flavonoid concentration (6).
In addition, the flavonoids of dark chocolate may also have greater biological effect than those of milk chocolate, because the calcium in milk chocolate may inhibit the flavonoid’s absorption in the intestines (6). Another suggestion is that milk proteins in milk chocolate may inhibit the activity of the antioxidants. This however, has been questioned since a recent study shows no differences in either antioxidant capacity or epicatechin bioavailability when milk chocolate was compared with non-milk chocolate, under isolipidemic and isocaloric conditions in healthy human subjects. The explanation of that might be that the kinetics of absorption changes by the food matrix, and it is possibly not dependent on flavonol-milk protein interactions (11).

![Flavonoids Diagram]

Figure 1. Structural skeleton and hierarchal classification of flavonoids (most common ones). *The predominant class of flavonoids found in cocoa and chocolate is flavanol. The figure is used after permission from the authors Eric Ding and Saket Girotra (6).

The dose-response bioavailability of chocolate flavonoids in humans has been shown to be positive (6).

There are many pathways the flavonoids take to act protectively against CVD (6). The mechanisms include the antioxidant, anti-inflammatory, anti-platelet and may be even by increasing the HDL levels, improving endothelial function and reducing blood pressure (6). Since the oxidation of LDL is central to the pathogenesis of atherosclerosis, the flavonoids act like antioxidants by their chemical structure that gives the compound free radical scavenging ability (6). Inhibition of LDL-oxidation was observed among healthy subjects, within 2 hours after intake of a flavanol-rich cocoa product. Long term feeding studies with flavanol-rich cocoa showed a total plasma antioxidant capacity increase and the reduction in the LDL susceptibility to ex vivo oxidation (11).

The antioxidative effect of catechins and epicatechin is attributed to the catechol group on the B ring, which can trap free radicals and chelate redox-active metals. This could be a possible explanation to the increased antioxidative effect being dependent on the increase of oligomer chain length, but also dependent on the nature of the antioxidant (11). Additionally,
flavonoids decrease lipid peroxidation of biological membranes by the intercalation of lipid soluble flavonoids into membranes of lipoprotein particles (6). Another mechanism that leads to a lowering in the initiation and progression of atherosclerosis is that of the catechins when suppressing microvascular endothelial cell production of IL-8, which is a potent chemoattractant for the atherosclerosis process (11).

Beside their atherosclerosis reduction effect, flavonoids (especially flavanols) may contribute to the inhibition of the oxidative conversion of NO to peroxynitrite and to the increase in the expression of NO-synthase (NOS) and NO-dependent vasorelaxation. Increased endothelial NOS expression and NO bioavailability have positive effects against endothelial dysfunction and thereby decrease blood pressure, slow down the atherogenic process and increase insulin sensitivity. The latter refers to that insulin sensitivity is partly dependent on the insulin-mediated NO-release (8).

As mentioned before, a special class of flavonoids is the flavan-3-ols (flavanols) that differ from the other flavonoids by appearing either as oligomers or esterified with gallic acid, in the aglycone form instead of glycosides. Flavanol-rich cocoa liquor for example stimulates NO production and on the other hand reduces other oxidative reactions (11). Reactive oxygen species (ROS) can be counteracted by antioxidants, which protect the heart. Cocoa antioxidants also constitute a protection against cancer. Suggested mechanism is the inhibition of phases of the cancer leading processes. The decrease in oxidation reactions means therefore a decrease in coronary heart disease and a number of cancer types (5).

Other in vivo studies show that flavanoid rich foods protect against oxidative damage in lymphocytes. Furthermore, chocolate components, especially catechins and epicatechins, have antiplatelet effects. Studies on healthy volunteers found that daily intake of cocoa beverages give a significant reduction in microparticle formation, activation markers and primary platelet activation as endpoints. Consumption of high procyanidin chocolate was found to reduce leucotrienes levels and decrease prostacyclin, in feeding trials. Leucotrienes are potent vasoconstrictors, proinflammatory agents that act as stimulants for platelet aggregation, whereas prostacyclin is a vasodilator that inhibits platelet aggregation (6).

Additionally, chocolate components inhibit lipoxygenase pathways. Lipoxygenase increase proinflammatory leukotrienes levels, which is an independent mechanism in atherosclerosis pathogenesis. Besides, cocoa polyphenols tend to decrease inflammation by inhibition of mitogen induced activation of T cells, reducing expression of interleukin-2 (IL-2) messenger RNA, polyclonal activation of B cells and reduction of IL-2 secretion of T cells. Chocolate procyanidins also have an ability to reduce inflammatory effects of cytokines like TNF-α and TGF-β by modulating these (6), and increasing the production of the anti-inflammatory cytokine IL-4 (11).

1.3.3. Theobromine in chocolate

Besides antioxidants like flavonoids and catechins, cacao is also a good source of theobromine, minerals like calcium, fluor, magnesium, sodium, copper and phosphorus, vitamins like A and B (riboflavin, thiamin, niacin) (9). Theobromine, a xanthine drug, is a bitter alkaloid that belongs to the class of the chemical compound methylxanthine, which includes theophylline and caffeine. It acts by stimulating the central nervous system causing an increased alertness (12). Theobromine’s impact on the human nervous system is lesser than
Theobromine is also found to be antitussive and inhibit cough by inhibiting capsaicin-induced sensory nerve activation in humans (14). It has an effect against asthma, and the suggested mechanism is relaxing the bronchi’s smooth muscles. By the inhibition of the phosphodiesterase (PDE) isoenzymes, the level of cyclic adenosine monophosphate (cAMP) increases; this in turn is attributed to the relaxant effect. There is evidence that the smooth muscle relaxation could affect a cAMP PDE. Another possible way of action is competitive antagonism of adenosine at adenosine receptors (12).

There are also negative effects of theobromine. For example it has an ability to cause gastrointestinal symptoms like anorexia, nausea and vomiting, nervousness, tremor and serious cardiovascular and CNS effects for plasma concentrations that exceed 200 µmol/l (12). Negative impact was also found on the reproductive indices of mice, caused by theobromine. The female reproductive capability was reduced, since a decrease in live pups per litter and pup weight was observed. Although, no changes in the percent of mice mating or litter delivering were found (13). However, theobromine is found in very small amounts compared to the other antioxidants (the procyanidins) in cocoa, which makes the contribution to the overall effect very small (5).

1.4. The new carbohydrate (NC)

The new carbohydrate (NC) is a disaccharide that is naturally occurring in honey, among others. The commercial product is enzymatically produced from another naturally occurring sugar (15, 16, 17, 18).

1.4.1. Characteristics of the new carbohydrate

NC is chemically classified as a carbohydrate which is non-cariogenic (toothfriendly), compared to sucrose. This is a result of that NC is hardly utilized or fermented by plaque bacteria in the mouth, inhibits the formation of insoluble glucans and prevents pH decrease in the mouth (15, 18). Streptococcus mutans is the bacteria involved in the development of dental caries. It is the main pathogenic microorganism in both experimental animals and humans. It acts by producing an extracellular enzyme, glukosyltransferas (GTase), which in turn catalyzes the synthesis of extracellular water-soluble and –insoluble glucans from sucrose. The synthesis of glucans advances the adherence of S. mutans to solid surfaces like teeth. S. mutans produces large amounts of acids from many different sugars, and the acids in turn damage to enamel surfaces by localized decalcifications (18).

The highest level of dental caries is shown to be promoted by sucrose compared to other mono- and disaccharides tested in animals. Furthermore, the Vipeholm study showed that the more frequent the sugar intake was, the more severe the dental caries was (18). This was specially associated with sweet between-meal-snacks. It was also shown that when sucrose-
rich foods are consciously restricted, dental caries also is reduced. NC on the other hand can not be fermented by certain strains of *S. mutans*, and this sugar even inhibits the production of insoluble glucans caused by *S. mutans*. This is demonstrated by using seven *S. mutans* strains that was subcultured in a broth medium containing 1% NC. Pathogen-free rats infected with *S. mutans* fed diet containing 56% NC did not develop significant dental caries. While rats infected similarly but fed a sucrose, fructose, glucose or a mixture of glucose and fructose containing diet significantly developed caries, non-infected rats (controls) did not develop significant caries when fed sucrose. The caries-inducing ability of glucose, fructose and the mixture of these was less than half of the sucrose's. Furthermore, when half of the sucrose content was replaced with NC, the caries development decreased compared with the development caused by the sucrose diet alone. When sucrose was 100% replaced with NC, caries development and plaque formation was reduced to the baseline levels. Although certain serotypes of *S. mutans* became adapted and fermented NC after repeated subculturing in broth containing NC, almost no oral streptococci was able to produce significant quantities of acid from NC in vitro (18).

NC has slower hydrolysis, slower absorption in the small intestine and slower glycemic response than sucrose. This results in lower and prolonged increase in blood glucose and insulin levels (figure 3), a result that is found in both healthy persons and diabetics. Very similar energy value is though obtained from both NC and sucrose (4 kcal/g) (15). It is completely hydrolyzed in the small intestine, but the hydrolysis rate is clearly slower than that of sucrose and maltose. In addition, intravenous studies show that any systemic NC can be hydrolyzed or excreted in urine (15, 16).

The taste of NC is similar to sucrose, but its sweetening power is much lower than that of sucrose (15, 16, 17, 19). It also has good characteristics like good heat and acid stability and low hygroscopicity. It also has viscosity in aqueous solutions that is similar to sucrose, but it is more stable under acidic conditions and melts at lower temperature in comparison to sucrose (160-185°C). Thus, the physiochemical properties of NC make the substance suitable as a sucrose substitute (15). NC is also a reducing sugar, the reason why it is involved in Maillard browning reactions (19).

Regular sugar (sucrose) often leads to high insulin levels, hypoglycaemic undershoot that may lead to earlier food initiation, in contrast to NC that may lower the risk of obesity. Diets with regular sugar seem to favor fat storage and may lead to insulin resistance (a pre-diabetic state) and obesity (20). NC is thus favorable in products for diabetics and those with prediabetic conditions like insulin resistance or metabolic syndrome (15, 20).

### 1.4.2. NC and the metabolic syndrome

Many studies have shown that hyperglycemia constitutes a risk factor for both micro and – macrovascular complications in type II diabetes mellitus (DM), but postprandial hyperglycemia also constitute a risk factor for cardiovascular diseases. Increased levels of postprandial plasma glucose (PG) may lead to equal or even more harmful effects than fasting hyperglycemia. Thus, the reduction in PG-levels leads to a delay in the development of cardiovascular complications. As a consequence of insulin resistance and impairment of early insulin secretion in response to an oral glucose load, postprandial hyperglycemia is developed. These complications can be reduced by reducing PG-levels, at least to start with.
However, subjects with postprandial hyperglycemia can control these parameters by a lifestyle intervention, which can be even more effective than drug intervention (3).

The beneficial effect of NC on the metabolic syndrome was examined in both rats and humans, by developing a NC based balanced formula. When the formula showed its positive effects in rats, the effects were examined in humans. A crossover study including twenty-three subjects with impaired glucose tolerance was conducted. The subjects were divided in two groups, one intervention to control (I/C) group and one control to intervention (C/I) group. For the first 12 weeks, the I/C group consumed foods that were 250 kcal less than their usual breakfast and instead replaced by 250 kcal of the formula, and for the last 12 weeks they consumed their usual breakfast as control meal. The C/I group followed the same scheme, but opposite in order, starting with the control meal. The study showed that consumption of the formula held back postprandial hyperglycemia as the 2-hours postprandial glucose levels decreased, improved the lipid profile such as FFA in serum in obese subjects and reduced visceral fat accumulation with the visceral fat area as indicator in humans. Consumption of the formula at breakfast not only shows an improvement of postprandial glucose levels after breakfast but the effect lasts even after lunch that does not contain the formula. This so called second-meal effect has been reported for consumption of low glycemic-index diet and it can be enhanced and prolonged if accumulated by long-term consumption of the formula. Long-term administration of the formula in rats improved insulin sensitivity and reduced visceral fat accumulation (3).

1.4.3. Toxicology

Regarding the toxicology of NC, no significant toxicological characteristics were found in several studies (15). When mineral status was examined in rats fed 30 % NC or 30 % sucrose (control group), no significant differences in mineral status, haematocrit and organ weights between the two groups were found (15). When rats were fed 30 % sucrose (control group) or 15 % NC for 13 weeks, neither changes in the earlier mentioned variables, nor toxicological changes were found. No signs of toxicity were found in rats when fed NC with levels up to 4.5 g/kg body weight per day. In addition, it is confirmed that survival, water and food intake and body weight were not affected by NC in another 13-week study in rats fed diets supplemented with 10% (8.1 and 7.0 g/kg body weight per day in female and male rats, respectively) NC and 10% sucrose (controls) (15). No abnormalities in growth, condition, haematology, immunotoxicology, neurotoxicology or microscopic and histopathological findings were observed. In addition, NC, in contrast to other low digestible carbohydrates, did not cause any caecal enlargement even at high concentrations. This physiological response is otherwise typical among rodents when fed high amounts of fermentable or osmotically active substances when entered the caecum (16).

No embryo- or teratogenicity was found in rats fed NC either. Dietary levels up to 10% (7 g/kg body weight/ day) NC showed no differences on body weight, weight of reproductive organs, food or water intake, reproductive performance and necropsy findings between the test and the control groups of pregnant rats. The rats’ general condition and behavior were checked everyday, from day 0 to day 21 of pregnancy, and during gestation water consumption and weight gain were measured. The pregnant rats were then autopsied and several parameters were measured, such as the weight of the ovaries, the gravid and empty uterus and live and dead foetuses (15, 17).
Human studies on healthy and diabetic subjects regarding gastrointestinal tolerance, insulin response, blood glucose and blood lipids were performed. The conclusion was that high doses of NC were similarly tolerated as sucrose. The overall insulin and fructose levels after NC ingestion were almost half those of sucrose. The serum levels of glucose were higher than after NC, but no significant differences between the areas under the glucose curve of any of the two substances were found at any dose level. In healthy subjects, plasma insulin and glucose levels after NC ingestion gradually increased and reached a plateau until 120 minutes. On the other hand, sucrose lead to a peak after 30 minutes of ingestion and the levels reached the initial level within 120 minutes. In type II-diabetics, the glucose and insulin levels increased more gradually than in healthy subjects and plateau was not reached. The insulin and glucose levels were though, just as in healthy subjects, significantly smaller than those after sucrose ingestion (15).

Regarding lipid metabolism, no significant effects were found between blood levels of LDL, HDL-C, VLDL an total cholesterol before and after NC ingestion in healthy subjects. The level of triglycerides increased though, but no significantly. These findings show that NC does not cause any gastrointestinal discomfort, even at as high doses as 50 g/day (15).

1.4.4. Applications and recommendations

Diets that are low insulinemic might initiate fat mobilization and the use of fatty acids as an energy source. Since NC is low insulinemic, it can be used in diet and for weight slimming tasks, according to the manufacturer (20).

Researches have shown that the consumption of diet based on high-carbohydrates and low glycemic/ insulinemic foods for long time may lead to reduced risk for diabetes, improves control of blood glucose in people suffering from diabetes (type II), lowers the risk for cardiovascular disease and can be used for weight control. FAO/WHO recommends that food manufacturers should be encouraged to expand low GI-foods to move forward prevention and treatment of such diseases (20, 21). In the European Union and Switzerland, there is approval for using NC in food in general (Novel Food Approval, July 25th, 2005). In Taiwan, South Korea and Japan NC is considered a food, and in the United States it is generally recognized as safe (GRAS) (20).

In summary, all of the earlier mentioned characters make NC a better energy source than regular sugars (20). This is even confirmed by The National Food Administration of Sweden by studies that prove that intake of diets containing high amounts of quick carbohydrates increase the risk for heart- and vessel diseases. Interesting to note here is that researchers have even found that diets containing high amounts of carbohydrates and low amounts of proteins was linked to lower death and cardiovascular diseases, while diets with high protein amounts and low carbohydrate amounts lead to the opposite effects (22).
1.5. The new fat (NF)

NF is a fat based on natural oil, which consists of omega-6 fatty acids, LCPUFA (long-chain polyunsaturated fatty acid) (figure 6) (23). PUFAs and monounsaturated fatty acids (MUFAs), constitute more than 90% of the oil. In addition to pinolenic acid, NF consists of linoleic acid and oleic acid (24, 25, 26).

There are a few studies done on NF and its satiating effect. Since there is a global epidemic rise in obesity, new strategies that are easy to apply for the general population, such as the use of natural appetite suppressants, are required. It has been shown that an overeating by 50 to 100 kcal above energy balance may lead to a gain of 1 to 2 kilos a year (25, 26).

1.5.1. Characteristics of the new fat

NF has been shown to control the appetite by significantly decreasing the levels of hunger by increasing the levels of the satiating peptide hormones cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1) (25). CCK is released from the epithelial cells of the duodenum when fatty acids and peptides from protein digestion come in contact with the duodenal epithelium (27). It stimulates secretion of pancreatic juice, regulates release of bile from gallbladder and regulates the contraction of smooth muscle in the gastrointestinal tract (4). GLP-1 is one of the gastrointestinal hormones that stimulate insulin secretion and it is produced in the ileum after passage of fatty acids and carbohydrates (11, 26). It is known to be a potent regulator of food intake by reducing the energy intake depending on the dose (26). Since both CCK and GLP-1 cause a delay in gastric emptying, early satiety and food consumption decrease is obtained (24). Intake of diets containing NF leads to significantly lower consumption desire and portion size. In turn, this reduces weight by decreasing energy and food intake, which means that a prolonged satiation feeling is obtained (23).

1.5.2. Mechanisms of action

Results show that NF can lead to decreased food consumption by increasing satiety hormones. There are suggested, but still unknown, mechanisms through which pine nut FFA and TG act to induce GLP-1 and CCK-8. The researchers think that the formation or the transport of chylomicrons might be included, due to the fact that only fatty acids that at least or more than 12 carbon atoms long that induce CCK-8. These long fatty acids are known to be transported from the enterocyte, directly by the portal vein and into the systemic circulation. There, the long chain fatty acids are absorbed as chylomicrons into the lymphatic circulation. The signaling paths of CCK-8 are related to transport of chilomicrons, therefore, this mechanism is favored since only fatty acids with ≥12 carbon atoms induces CCK-8. This might mean that pine nut FFA and TG may have an effect on chylomicron formation or transport, which in turn may influence CCK-8 release. Satiating signals are then sent to the brain by the fast CCK-8 release that activates the CCKα receptors of the afferent fibres on the nervus vagus,
causing reduction in gastric emptying and thereby increasing satiety feeling, decreasing appetite and limiting of food intake (26).

1.5.3. Applications and recommendations

According to the producer of NF, recommended daily intake of NF is 3 g, 30 – 60 minutes before meal with the highest energy intake, if the aim is to reduce the regular meal size. If the aim is to reduce snacking instead then 3 g is recommended in between meals, and to help slimming then 3 g is recommended in meal replacer. The maximal daily dose is 6 g a day though. NF applications may include dairy (e.g. milk drinks, fermented and non-fermented yogurt drinks, soy drinks), bakery (e.g. biscuits, cookies, bread, bars, cereals), beverages (juices, soft drinks, sport drinks), sauces, soups and others. It is easy to incorporate oils in foods by homogenization, and oils can withstand pasteurization and ultra high temperatures (UHT). The shelf life of UHT yoghurt drink with NF is up to six months. Up till now, there are only prototypes existing, excluding the first product containing NF which is newly released in the food market in Great Britain. In EU, NF is not classified as a Novel Ingredient because of history of use. However, in the United States it is classified as a self affirmed GRAS (Generally Recognized As Safe), because of the long history of safe use of the oil and the oil source (23).

1.6. Glycemic index (GI) and glycemic load (GL)

There are myths about how fast carbohydrates are digested in the body. One of those myths says that complex carbohydrates are always digested slower than simple sugars. Now we know that so is not the case as there are many factors that influence carbohydrate metabolism and glucose response. The glycemic index (GI) is a method to rank foods on a scale according to the fact of how foods raise blood glucose levels after intake. Carbohydrates that are digested rapidly have the highest GI value, which means that these foods have high and fast blood glucose response. That also means that carbohydrates which break down slowly have low GI value and release glucose gradually into the bloodstream (28).

Foods that have 44 or less as a GI value are classified as low glycemic foods, a value of 56 – 69 is a medium GI value and values of 70 or more are classified as high GI values (28). The glycemic load (GL) refers to the sum of each food item’s GI and the carbohydrate amount in a food portion (30). The idea is that foods with high GI and consumed in small amounts give the same effect on blood sugar as larger amounts of foods with low GI (30).

1.6.1. The usage of GI and GL

There are different opinions about the classification of foods according to their glycemic responses, regarding its clinical benefits. For example, methodological variables like the food-portion size, the standard food chosen, repeated standard food testing, frequency and length of time of the sampling of blood, how the area under the glycemic-response curve is calculated and subject characteristics like sex, age, weight and glucose tolerance status affect the analysis of glycemic responses and the obtained GI-values. Despite this, recent studies support the idea that the use of GI might be beneficial. When carbohydrate intake with high GI-foods increase, blood glucose, triglyceride levels and insulin may also increase. In contrast
to that, when carbohydrate intake with low-GI starchy foods increase, carbohydrate intake may be increased without the mentioned negative effects (31).

To determine whether the differential impact on blood glucose and insulin responses induced by contrasting GI foods does not differ when provided in portions with different sizes, a study including 12 obese women was carried out. In random order, the participants received two meals with high GI (86-91 %) and two meals with low GI (40-43 %). The two meal types where given as two meal sizes with energy supply equal to 23 % and 49 %. After blood sampling over a 5-hours period, it was shown that serum glucose and insulin incremental area under the curve over 2 hours were almost twice as big for the high GI meals as that of the same sized low GI meals. On the other hand, when the two meals with similar GL but different types and amounts of carbohydrate were compared to each other, serum glucose and insulin responses were largely the same. Glycemic load thus was useful in forecasting the acute impact on blood glucose and insulin responses within the perspective of mixed meals. No significant differences for the serum glucose response were observed after the small meal intake. However, the conclusion was that when different amounts of carbohydrates are consumed, GI alone is not useful to predict the glycemic impact. Thus, the GL was supported to differentiate the acute impact on blood glucose and insulin responses as long as mixed meals were consumed. However, the results of blood glucose and insulin responses in this study were similar to those found in other studies using isolated carbohydrates. This shows that in spite of that mixed meals contains other macronutrients, there is a scarcely noticeable effect of these on the blood glucose and insulin responses (29).

1.6.2. Classifications of GI-foods

Another myth says that foods low in carbohydrates also are low in GI values, which is wrong. For example at 25 % carbohydrate level, GI may be between 10 and 95. There are many factors that influence the glycemic response, the nature and amount of carbohydrates, the nature of the starch (amylase, resistant starch, amyllopectin), the nature of the monosaccharide components (glucose, fructose, galactose), food processing or cooking (the degree of starch gelatinization, particle size, cellular structure) and other food components (fat, protein, antinutrients, organic acids, dietary fibre). For example the GI value of white pasta (GI = 55) differs from that of white pasta with tomato sauce (GI = 35). Factors like presence of other specific nutrients in foods influence starch digestion by affecting stomach’s emptying and viscosity of gut contents (28).

Meta analysis of randomized controlled studies has shown that foods with low GI values might be helpful for people with diabetes in both type I and II. Individuals that followed low glycemic diets showed clinically relevant reduction in blood glucose levels (33). To avoid obesity, energy balance must be maintained. Energy balance is achieved if the energy intake is equal to the energy consumption. If the energy intake is greater than the energy consumption there is an imbalance, which leads to obesity. Studies have also shown that low glycemic foods might be useful in diet intervention for individuals suffering from the metabolic syndrome (28).
1.7. Dietary fibre

Plant carbohydrates that do not break down in the digestion and reach the large intestine largely unchanged are used to be called dietary fibers. The biggest part of the dietary fibers is found in vegetable cell walls that mostly consist of cellulose, hemicelluloses, lignin and pectin. It is found in fruits, wholegrain meal, lenses, vegetables, root vegetables and dried peas and beans (34).

1.7.1. Characteristics of dietary fibre

Dietary fibre is important for a normal functioning intestine. Many of the fibers bind water so the volume of the feces increases and become smooth. Thus, fibre-rich foods are recommended against constipation. Foods rich in dietary fibre give a higher fullness feeling and thereby lead to decreased snacking. This is because those foods need more chewing, give higher saliva release and bind water in the stomach. In the large intestine, the bacteria have an ability to utilize the dietary fibre and after breakdown pre-ferment their breakdown products in the absence of oxygen (35).

Also relevant is that fibre-rich foods lead to a slow rise of the blood sugar levels. Certain so called gel-forming dietary fiber types like pectin in fruits and vegetables can even reduce the blood cholesterol levels. High levels of dietary fiber can on the other hand lead to gastrointestinal problems such as gas formation and diarrhea (34).

During the end of the 1990s, it was observed that dietary fibre, especially gel forming fiber, can prevent coronary vessels disease. The hypothesis may origin from Hugo Trowell, an internal medic, and Denis Burkitt, a surgeon, who around the 1970s introduced the idea that dietary fiber might prevent certain age related degenerative diseases. Both have, during a period of more than 20 years of clinical work in Uganda and Kenya, noticed that the widespread diseases of the Western world largely do not exist in the original population. There are a few papers that explain the relationship between dietary fiber and cardiovascular disease (35).

An independent predictor of future cardiovascular disease, the metabolic syndrome and diabetes mellitus is the high sensitivity C-reactive protein (CRP), which is a marker of acute inflammation. The consumption of dietary fiber is associated with reduced lipid oxidation; that, in turn, lead to reduced inflammation. Since there is a possibility to modify the concentrations of this protein in serum through diet, there also is a possibility to influence the risk of these diseases. The relationship between dietary fibers and the serum levels of CRP was examined in a study with healthy subjects. Factors including measurement of CRP concentrations, diet and a number of other factors were collected at baseline and every 13th week over a 1-year period. Thereafter, body composition, diet, blood, physical activity and psychosocial variables were collected every visit of a total of five visits. The study showed an inverse relationship between serum CRP concentrations and dietary fiber intake (soluble and insoluble fibers, separately). The likelihood for increased CRP concentrations was 63 % lower in subjects with the highest quartile of total fiber intake than those in the lower quartile (36).
There are other studies that have found a relationship between dietary fibre intake and other diseases. To mention, there is evidence that better lung function and reduced prevalence of chronic obstructive pulmonary disease (CORD) and dietary fibre intake (37). There are also beneficial effects of dietary fibre in decreasing the risk of colon cancer by sodium butyrate induction of Smad3 (a protein in that helps tumor preventing (38) and signaling of transforming growth factor (TGF)-β and its tumor suppressor activity in the epithelial cells of the gut (39).

1.7.2. Recommendations

Recommended daily intake of dietary fiber by The National Food Administration of Sweden (SLV) is 25-35 g for adults, which is equal to ca 3 g/MJ (40). Due to the relevance of dietary fibre, it was decided to include dietary fibre in our wafer chocolate products.

1.8. Fat reducing protein material (FRP)

Since the fat content in the final products should be as low as possible, a new ingredient used to reduce fat in chocolate products was of interest.

The ingredient is a fat reducing protein material (FRP), a light yellow dried protein material in a powder form, and it is extracted from partially hydrolyzed collagen that is derived from animal tissues with characteristic flavor and odor. It has properties that make it able to replace cocoa butter in chocolate masses, without modifying their physical characteristics, such as viscosity, crystallization limit or draining, or the sensorial characteristics, such as flavor and melting point. It is classified as a GRAS (generally recognized as safe) (41, 42).

1.8.1. Applications and recommendations

FRP was developed to be used in applications for fat-reduced milk chocolates by decreasing the overall and saturated fat contents. As it lowers the fat content, it increases the protein value in the chocolate since it is a protein (43), which favors the plans for the final product. Applications involving FRP are chocolate coatings, fat based fillings for wafer and cookies, energy reduced chocolate products and hazelnut and chocolate spreads (43).

FRP can be used to develop milk chocolate products that are fat reduced up to 30 % with even lower energy content. Higher protein content is also obtained since there is less cocoa butter and more milk solids and proteins from FRP. The resulting product has a viscosity similar to that of the standard recipe. FRP neither alters the process conditions remarkably nor interferes in the cocoa butter crystal formation. Taste, melting, hardness and mouth feel in fat reduced chocolate with FRP are close to those of regular high fat chocolate (43).
2. MATERIALS

- Raw material No. 1 Protein (R1) (D.M. > 82 %)
- Raw material No. 2 (R2) (Fat content 55%)
- Raw material No. 3 Fat (R3) (Purity 99.9 ±0.5 %)
- Raw material No. 4 (R4) (26 % fat, 26 % protein)
- Raw material No. 5 carbohydrate (R5) (purity 99)
- Raw material No. 6 Fat (R6) (Purity 100 %)
- Raw material No. 7 Fat (R7) (solid fat content is 74 % at 20 °C.)
- Raw material No. 8 Fat (R8) (Purity 94 %)
- Raw material No. 9 Fat (R9) (Purity 100 %)
- Raw material No. 10 Protein (R10) (D.M. 99 %)
- Raw material No. 11 Dietary fiber (R11) (D.M. 96±1 %)
- Raw material No. 12 Sugar alcohol (R12) (Purity 99.5 %)
- Raw material No. 13 Fruit concentrate (R13) (Purity 92 g fruit/ 100 g finished product)
- Raw material No. 14 Protein (R14) (Purity 84-92 %)
- Raw material No. 15 Fat (R15) (Purity 100 %)
- Common salt (NaCl, 99 %)
- Sodium bicarbonate (Purity > 99 %)
- Raw material No. 16 (R16) (D.M. 85.5 %)
- Raw material No. 17 (R17) (D.M. 89 %)
3. METHODS

3.1. Formula development

Two different wafer chocolate products, between meal snacks, were developed in this study, in aim to choose the ultimate product in the future. One of them contained NF and the other lacked it, otherwise the products were identical. In both products, sucrose which is usually used in regular chocolate masses is completely substituted with NC. However, a wafer chocolate product is made of wafers, a filling between the wafers, and finally dipped in chocolate. In order to create a formula for the products, another independent wafer chocolate product (a new sport product) was first made only to learn how to make a chocolate product, including using the machines and to understand the context of the ingredients that are usually used to manufacture a wafer chocolate product. The new sport product was made in two variants, one dipped in milk chocolate and one in dark chocolate. The new sport product was not used in this study.

After making and studying the formula of the new sport product (see the next section), it was decided that the wafer formula should be similar to that used in the between meal snacks because it is fibre-rich. Since the product was new, small changes in the amounts of the wafer ingredients were made, to make the optimal wafer. On the other hand, the fat content in both the milk chocolate and the dark chocolate masses of the new products was increased, due to high viscosity caused by the protein in the original recipe.

Both of the wafer chocolate products were covered with milk chocolate for two reasons. First, it is easier to adjust the fat content in milk chocolate than in dark chocolate. This would match the goal of making final products with as low fat content as possible. Second, since milk chocolate has higher protein value (from milk) than dark chocolate, the final protein percentage share would be closer to the desired level. From this point of view, less fat and more protein were more favored than the higher antioxidant content found in dark chocolate. To further reduce the fat content of the milk chocolate, a new ingredient, FRP, aimed at replacing a part of the fat in chocolate applications by reducing the viscosity was used.

The new sport product contained a fruit based protein-rich filling. However, this filling does not match the goal of the between meal snacks, since the fruit concentration was too low and the protein value was too high. Even the protein value in the new sport products was too high so it was adjusted (lower) in the between meal snacks.

The amount of NF in the between meal snacks was decided based on recommendations from the producer. It was planned that a part of NF would be put in the chocolate mass and the rest in the fruit filling. The planned amount of fiber was 5-6 g/100 g, based on Cloetta Fazer’s own idea of what a relevant amount should be. Dietary fibre was put in the fruit filling.

Based on the nutrition recommendations of The National Food Administration of Sweden, the daily energy recommendations, including those for intake of protein, carbohydrate and fat was set as a goal for the final products. According to recommendations for how large part of the energy daily consumed should come from snacks, and assuming that two snacks a day are
eaten, the total energy content of each macronutrient for the product was calculated. This is illustrated in the table I-III (p 1.1). The approximate required energy intake for a snack is between 69 and 411 Kcal (average 240 Kcal), of which 38 – 226 Kcal is carbohydrates, 21 – 123 Kcal is fat and 10 – 62 Kcal is proteins.

3.2. Preparation of the milk chocolate mass

The machines used in manufacturing test products were in principal the same machines used in for manufacturing the products in the fabric, but in a laboratory scale. To start with, the formula of the milk chocolate sport product (“the original formula”) was studied and changes were done. First, only sugar was adjusted, milk chocolate with 50 % sucrose and 50 % isomaltulose was made. This was then adjusted many times so the final formula was totally different from the original formula.

3.2.1. Mixing

The ingredients to be mixed (Sugars, R2, a part of R3, R4 and R7) were weighted and put in the conching (mixing) machine, a machine for homogenization and vaporization of water from chocolate masses (figure 7), Varimixer Bjørn, 91 RN 20, Wodschow & Co. Denmark. Temperature was controlled by the help of a thermostated water bath.

The raw materials were mixed in the mixing machine for approximately 50 minutes. In later experiments, a big part of R7 was replaced with NF. R1 and R10 were added later to give the final concentrations of 13 % per weight and 5 % per weight, respectively.

The mixing time differed depending on when the raw materials were enough mixed to a consistent appropriate for refining, the next step in chocolate preparation. The ingredients were mixed at 40 ºC, except when the formula contained FRP, where the recommended mixing temperature was 60 ºC (43).
3.2.2. Refining

The refining step required correct settings and temperature. It was either done in one or two steps, usually two. The first step concludes rough refining and it is usually followed by the fine refining step. An overdone fine refining might lead to a burning sweet taste, while an overdone rough refining might lead to a sandy feeling in the mouth. The chocolate dough to be refined passes through 3 rolling cylinders with specific adjustable distance in between depending on the desired fineness of the product, and they roll in opposite direction to each other. The product after the refining step is obtained in powder form. A principal sketch of the refining machine is seen in figure 8.

In this study, the chocolate mix was almost always refined in two steps depending on the particle size of the final product. In both refining steps the temperature of the mid cylinder was adjusted to 40 °C and the other two to 20 °C. After the refining the particle size of the refined product was measured by a simple instrument with two small walls between which a small oil-diluted test sample was put. The walls were manually moved towards each other till it was no longer possible to move any further, otherwise the particles would be damaged. The diameter of the particles of the sample was digitally displayed. The diameter was then compared to the usual size of such product. The particle size varied depending on what chocolate mass was manufactured.
3.2.3. Conching

Two variants of chocolate masses were made, one where a part of cocoa butter was replaced with FRP, and one with no replacement.

The refined chocolate was followed by conching, further working up the powder to obtain the desired taste and texture. During conching, taste substances that are not wanted disappear together with water. The ingredients of the mass nebulise and better taste and consistence appear. The temperature and the time of the conching vary depending on what chocolate mass is manufactured.

The remaining part of R3 was added to the powder and put in the conching machine (figure 7). The conching temperature was the same as used in the mixing (40 °C), except when FRP was added in later experiments, where the temperature was adjusted up to 60 °C according to the recommendations from the manufacturer. The conching process was run for 5 hours. After 4 hours, soy lecithin was added to the mass then the conching was continued till the last hour. R8 (an emulgator) is usually added mainly to lower the viscosity. R9 is another emulgator that was added to FRP- containing chocolate masses, in later experiments. If the chocolate mass was too viscous a short while after R8 was added, more of R3 (fat) was carefully added till the texture looked more proper. The finished product was stored in a heat cabinet (60 °C) until it was used.

After conching, the viscosity of both variants of chocolate masses was measured by a viscosity machine, Anton Paar, Physica MCR xx1 Series, Anton Paar, Germany, GmbH; No considerable differences in viscosity were found. FRP reduced the fat content with 26 %, why it was used.
3.3. Preparation of the fruit filling

The fruit filling used was an apricot concentrate (92 g apricot/ 100 g finished product) with a very low water content. It is an ochreous yellow colored very thick paste. To soften the paste, it was heated in a microwave oven on full effect a few seconds till it was soft enough to spread on a wafer, but not heated till it boiled. If the filling starts to boil, its color would change due to water loss and that would alter its properties.

Dietary fiber was added to both of the NF-containing and the NF-lacking fruit filling. NF was only added to the product that was aimed at containing NF. Because the fiber was hard to melt, it was first added to a microwave-heated liquid sugar alcohol (R12), mixed, and the mix was then added to the heated apricot paste and mixed again. The total fiber value in the paste was 5-6 g/ 100 g finished product. NF was, at the expense of the apricot paste, added to the microwave heated apricot paste. Different amounts of NF were added till it was not proper to put more, because then the paste would be too fatty. The energy distribution of the fruit filling is shown in table IV.

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>NF-containing fruit filling Energy/ 100 g filling (Kcal)</th>
<th>NF-lacking fruit filling Energy/ 100 g filling (Kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>151</td>
<td>163</td>
</tr>
<tr>
<td>Fat</td>
<td>42.4</td>
<td>11.1</td>
</tr>
<tr>
<td>Protein</td>
<td>9.7</td>
<td>9.84</td>
</tr>
<tr>
<td>Total</td>
<td>203</td>
<td>184</td>
</tr>
</tbody>
</table>

3.4. Preparation of the wafers

R16, R17, salt, R15, sodium bicarbonate and water was weighed in a basin then mixed by a whisk till the ingredients were well mixed. A small amount at a time, just over 2 dl of the mix, was spread over a 175 °C heated, wafer patterned iron surface, and then pressed with another iron block of the wafer baking machine, Franz Haas Austria, HZ-WA. Figure 9 shows a principal sketch of the machine. Initially, in the original formula, the baking time was 2 minutes, but this was adjusted to 1 minute and 45 seconds, because the wafers broke otherwise. The product was a brittle “waffle” with low moisture content. It is important that the moisture content is as low as possible so the brittleness can be maintained. When the wafers were not used directly after baking, they were wrapped as tight as possible in a special plastic foil, by a lamination machine, to keep them as fresh as possible for later use.
3.5. Making the products

First, the goal was to make products in a structure of Cloetta’s regular wafer chocolate, which means 3 wafer layers, filling between the wafers and a chocolate overlay. Later, even products with a shape of the sport product were made and compared with the products with the shape of Cloetta’s wafer chocolate, in regard to the nutritional content. The sport product is made of three wafer layers, filling between the wafers and then dipped in chocolate. The chocolate value in relation to the wafers and the filling is much higher than that of Cloetta’s wafer chocolate. After the nutritional comparison it was decided that the products would have similar structure to that of Cloetta’s wafer chocolate, but with one extra wafer and filling layer.

3.5.1 The products with chocolate overlay

Filling was heated a few seconds in the microwave oven until it was soft enough to be spreadable on wafers. Carefully, filling was spread on one wafer to a thin even layer, next wafer was put on the filling layer and a new layer was spread and so on. The obtained wafer-filling block of 29×23 cm was then divided in smaller pieces of ca 7×3.7 cm. The finished chocolate mass was taken out from the heat cabinet, mixed a few minutes with a whisk then moved over a special table to temper. This process is called tempering or pre-crystallization, and it is done to obtain a chocolate product with right characteristics. During the tempering only the chocolate fat is influenced. This means that the chocolate is cooled down and pre-crystallized so it stiffens in the right way during the product forming. This is important since if the chocolate is not tempered the chocolate would be less shiny, maybe have some degree of grayness and a coarse consistence.
Almost half of the chocolate mass was spread over the tempering table, stirred and put back and stirred into the remaining chocolate mass in a basin. Temperature was measured and the tempering process was repeated till the right temperature, 28-29 °C, was reached. The wafer-filling pieces were dipped in the tempered chocolate, moved and put on an iron bar and the chocolate layer was blown with an air blow gun to remove some of it so an enough thin and even layer was obtained. The pieces were then put in the freezer and taken out after about one hour. The finished product was obtained.

3.5.2. The chocolate-dipped products

The following method of making chocolate products used a plastic form in which the products were shaped into uniform size.

Wafer-filling blocks (29×23 cm), were made as described above (p. 3.5.1.). The block was then divided in smaller pieces of 3.7×1.7 cm, consisting of 3 layers of wafers and two layers of filling. The chocolate mass was tempered to 28-29 °C. The plastic form (same used to prepare the sport product) was then heated a few seconds with hot air and then put on a shaking table. The shaking made air bubbles float and leave the chocolate surface. Gradually, the tempered chocolate was poured out into the form. The small pieces of wafer-filling blocks were thereafter immediately put in the form so they were à demi dipped in chocolate. A few seconds later more chocolate was poured out on top of the dipped pieces so they were completely covered. The shaking was still on so the chocolate floated up and filled the whole form. The shaking was turned off and the form was let to cool down till the chocolate stiffened. The form was put in the freezer and taken out about an hour later. Thereafter, the form was turned upside down and hit a few times against a table surface to let the finished pieces out.

3.6. Test products and placebo

The placebo used in the study was 30 g of Cloetta’s regular wafer chocolate. Test product number 1 contained 5 g/100 g NF finished product, which was equal to 1.5 g NF in the 30 g finished product. Test product number 2, also 30 g, contained no NF. The test products were the ones developed in this study. Placebo contained twice as much energy as the test products.

3.7. Subjects

Seventeen healthy test subjects, 7 women and 10 men, with ages between 18 and 30 years (average age 20.6 years) of age were selected based on their health declaration (appendices 1). The BMI of the subjects was between 18.4 and 27.7 Kg/ m² (Average: 20.9 Kg/ m²).
Inclusion criteria were absence of any cardiovascular diseases, serve blood deficiency, diseases in stomach, intestines or other gastrointestinal organ, diabetes mellitus of any type, leukemia, hepatitis, too low/high blood pressure, eating disorders and not using medications.
Two subjects withdrew after the first testing day, and another withdrew after two days, due to personal reasons. Fourteen subjects completed the study.

3.8. Experimental design

Subjects were asked to avoid any strenuous physical activity for 2 days prior to the testing days, and they had to avoid eating and drinking (except water) for at least 3 hours prior to the testing hour. After arrival they ate a product of 30 g followed by drinking a glass of water, and blood glucose from arm vein was measured and analyzed within a few seconds by Hemocue Glucose 201 (accuracy: correlation of r>0.98 when compared to other laboratory methods; precision: SD <0.3 mmol/L (6 mg/dL)) at baseline, 20, 40, 60, 80, 100, (110), 120 and, only at the first study day, 134 minutes after product intake. Measurements were continued until the blood glucose response returned to baseline. All measurement was carried out by two nurses. A form containing questions regarding the level of hunger, the taste opinion, the level of happiness after eating the products and the possibility of buying the product in the future, was distributed to the participants (appendices 2) and then collected at the end of the study. At the first testing day subjects ate a 30 g product containing 1.5 g NF, 1.6 g fiber, 54 % energy as carbohydrates, 36 % as fat and 10 % as protein. The total energy content was 129 Kcal. The next testing day, four days later, they ate placebo (Cloetta’s chocolate product), 30 g. It contained 8 % energy as protein, 46 % as fat and 47 % as carbohydrates. The total energy content was 257 Kcal, which was twice as much energy as the test products. The last study day, 2 days later, the subjects ate 30 g product containing no NF, 1.6 g fiber, 59 % energy as carbohydrates, 31 % as fat and 10 % as protein. The total energy content was 125 Kcal. Table V shows the energy value of each macronutrient in each product of 30 g.

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>Placebo (Kcal)</th>
<th>NF-containing product (Kcal)</th>
<th>NF-lacking product (Kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>120</td>
<td>69.2</td>
<td>72.3</td>
</tr>
<tr>
<td>Fat</td>
<td>117</td>
<td>47.2</td>
<td>39.4</td>
</tr>
<tr>
<td>Protein</td>
<td>20.0</td>
<td>12.9</td>
<td>12.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>257</strong></td>
<td><strong>129</strong></td>
<td><strong>125</strong></td>
</tr>
</tbody>
</table>
4. RESULTS

4.1. Result analysis

The blood glucose values are illustrated in a diagram, where the x-axis represented the time in minutes and the y-axis represented the rise in blood glucose levels in mmol/L. The results from the 3 study days for each person were combined in one diagram, to make it possible to compare the products and the placebo to each other. First, analysis was focused on each subject’s blood glucose response based on the maxima of the blood glucose levels, the evenness of the curves and when the baseline levels were reached. High peaks mean high rise of blood glucose levels, even curves mean a slow rise of blood glucose levels and an early return to baseline levels means rapid digestion of the product’s carbohydrates (fast carbohydrates). Results for S15 – S17 were not included in the analysis. Second, the mean values of all the subjects’ blood glucose responses together were plotted against the time in one diagram and the result was analyzed. The analysis were based on the earlier mentioned characteristics (peak, fluctuations, evenness), but also on the incremental area under the obtained curves and the statistical significance of the mean values between the products at each time point. The area under the curve (AUC) was measured by the formula below:

\[
iAUC_{1,n} = \frac{At}{2} + At + (C - B)t/2 + Ct + (D - C)t/2 + Dt + (E - D)t/2 \ldots \text{etc. (31)}
\]

where A, B, C, D and E represent positive blood glucose increments, t is the time interval between blood samples. If increment D is positive and (greater than baseline) and E is negative (less than baseline), only the area between D and E above the baseline was included (31).

The statistical significance was measured by a one-way ANOVA using the means and the SD values of the 14 tests.

4.2. Results

Fourteen subjects completed the experiment, two subjects withdrew after the first day and one subject withdrew after the second day. No illness was observed or reported.

4.2.1. Blood glucose levels

The obtained blood glucose curves are shown in figure 10. It is worth to mention that the baseline blood glucose levels for the same person were different from each other at the three different study days, and this was notable for all the subjects. The baseline levels are shown in table VI, where the NF-containing product was eaten at day 1, placebo at day 2 and the NF-lacking product at day 3. However, five of fourteen (36 %) placebo-curves showed the highest peaks (blood glucose maxima) compared to the two other product-curves of the same person (referring to S1, S2, S3, S11 and S13). Same number, 36 %, was obtained from the no-NF
curves compared to the other curves (S4, S8, S10, S12 and S14). Three (21 %) NF curves showed the highest maxima (S5, S6 and S9).

Thirteen placebo curves of fourteen (93 %) showed rapid fluctuations of blood glucose levels compared to the other product curves of the same person (S1, S2, S3, S4, S5, S6, S8, S9, S11, S12, S13 and S14). Ten (71 %) NF curves showed rapid fluctuations of blood glucose levels (S1, S2, S4, S5, S6, S7, S9, S10, S12 and S14), while 9 (64 %) no-NF curves showed similar fashion (S1, S2, S4, S8, S9, S10, S12, S13 and S14). Three (21 %) NF curves showed fair evenness (S3, S11 and S13), while 1 (7 %) no-NF curve was fairly even (S5). No placebo-curve showed any clear evenness, but 1 (S7) showed weak fluctuations compared to the other curves of the same person.

The mean values of all the subjects’ blood glucose responses for the three study days are shown in figure 11. Placebo showed the highest blood glucose maxima, followed by the NF-containing product and the NF-lacking product. The NF-lacking product had a maxima that was less than half of that of the NF-containing product and 1/3 of that of placebo. Furthermore, the blood sugar levels generated by the NF-containing product returned to baseline slightly earlier (after 108 min) compared to placebo (after 112 min), and earliest were the levels generated by the NF-lacking product (after 70 min).

There was no significant statistical significance (p> 0.05), by one-way ANOVA, between the mean values for the 14 tests at respective time points and snack type (table VII). The incremental area under the curves was calculated to approximately 26.42 mmol.min/L for the NF-containing product, 34.76 mmol.min/L for placebo and 19.66 mmol.min/L for the NF-lacking product.
Figure 10. Blood glucose responses obtained after oral administration of placebo, the product containing NF and the product lacking NF. S1 refers to Subject No. 1, S2 refers to Subject No. 2 etc.
Figure 11. The summary of all the subjects’ results of the blood glucose responses for the tree study days. The standard deviations for each mean value are given in table VII.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 (W)</td>
<td>5.7</td>
<td>6.2</td>
<td>8.0</td>
</tr>
<tr>
<td>S2 (W)</td>
<td>5.2</td>
<td>5.6</td>
<td>5.9</td>
</tr>
<tr>
<td>S3 (W)</td>
<td>5.3</td>
<td>5.0</td>
<td>6.2</td>
</tr>
<tr>
<td>S4 (M)</td>
<td>4.6</td>
<td>5.1</td>
<td>4.0</td>
</tr>
<tr>
<td>S5 (M)</td>
<td>4.9</td>
<td>5.4</td>
<td>5.0</td>
</tr>
<tr>
<td>S6 (W)</td>
<td>4.8</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>S7 (M)</td>
<td>5.5</td>
<td>5.3</td>
<td>6.1</td>
</tr>
<tr>
<td>S8 (W)</td>
<td>6.0</td>
<td>4.9</td>
<td>4.9</td>
</tr>
<tr>
<td>S9 (M)</td>
<td>4.5</td>
<td>5.3</td>
<td>4.8</td>
</tr>
<tr>
<td>S10 (M)</td>
<td>4.8</td>
<td>5.1</td>
<td>5.3</td>
</tr>
<tr>
<td>S11 (M)</td>
<td>3.5</td>
<td>4.2</td>
<td>5.0</td>
</tr>
<tr>
<td>S12 (M)</td>
<td>6.0</td>
<td>6.3</td>
<td>6.1</td>
</tr>
<tr>
<td>S13 (M)</td>
<td>5.6</td>
<td>6.0</td>
<td>6.1</td>
</tr>
<tr>
<td>S14 (M)</td>
<td>5.5</td>
<td>5.8</td>
<td>5.3</td>
</tr>
<tr>
<td>S15 (M)</td>
<td>5.0</td>
<td>5.3</td>
<td>-</td>
</tr>
<tr>
<td>S16 (W)</td>
<td>5.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S17 (W)</td>
<td>3.6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

W = Woman, M = Man
Table VII
The mean values and the standard deviation (SD) of the blood glucose levels at respective time points. Used formula: \( SD = \sqrt{\frac{\sum (x-\mu)^2}{n-1}} \), where \( x \) is the value from the sample, \( \mu \) is the mean value (mmol/L) and \( n \) is the number of \( x \). The time is given in minutes and illustrates after how long time, starting from baseline (time=0), the blood glucose levels were measured.

<table>
<thead>
<tr>
<th>Time</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>110</th>
<th>120</th>
<th>130</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu (+NF) )</td>
<td>0</td>
<td>0.44</td>
<td>0.71</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>-0.05</td>
<td>-</td>
<td>0.10</td>
</tr>
<tr>
<td>SD</td>
<td>-</td>
<td>0.68</td>
<td>0.66</td>
<td>0.60</td>
<td>-</td>
<td>-</td>
<td>0.60</td>
<td>-</td>
<td>0.50</td>
</tr>
<tr>
<td>( \mu (Placebo) )</td>
<td>0</td>
<td>0.04</td>
<td>0.87</td>
<td>0.37</td>
<td>0.22</td>
<td>0.18</td>
<td>0.04</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>-</td>
<td>0.28</td>
<td>0.50</td>
<td>0.62</td>
<td>0.63</td>
<td>0.50</td>
<td>0.61</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>( \mu (No NF) )</td>
<td>0</td>
<td>0.20</td>
<td>0.33</td>
<td>0.29</td>
<td>-0.17</td>
<td>-</td>
<td>-0.15</td>
<td>-</td>
<td>-0.18</td>
</tr>
<tr>
<td>SD</td>
<td>-</td>
<td>0.59</td>
<td>0.93</td>
<td>0.80</td>
<td>0.71</td>
<td>-</td>
<td>0.57</td>
<td>-</td>
<td>0.15</td>
</tr>
</tbody>
</table>

4.2.2. Hunger levels

The hunger levels, illustrated in figure 12, did not show any helpful trend. 6% of the subjects felt hunger within the first half hour after eating NF- containing product, while none felt hunger after eating the NF- lacking product and 19% after eating placebo, within the same period. The second half hour, 38% of the subjects felt hunger after eating the NF- containing product, which was equal to 21% after eating the NF- lacking product and 31% after eating placebo. Within the third half hour, 26% felt hunger after eating the NF- containing product, 29% after the NF- lacking product and 13% after placebo. The last half hour, 6% felt hunger after eating the NF- containing product, while none felt hunger after eating the other two products. However, this means that subjects felt least hunger after eating the NF-lacking product (50%), compared to placebo (37%) and the NF-containing product (25%).
4.2.3. Taste opinion and future market

Regarding the taste opinion, less subjects liked the taste of the healthy products compared to placebo. 25 % liked the taste of the products, 44 % did not like it very much and 31 % did not like it at all. 87 % liked the placebo and 13 % did not like it very much, but none disliked it. When subjects were asked to write down what other tastes they preferred, the answers was vanilla, orange, banana, berries, strawberry, more chocolate taste, nougat, cappuccino and cola, starting with the most wanted taste. However, it is important to emphasize that the focus was not directed on the taste in this study, since the effects were more important.

Despite that, 57 % of the subjects could imagine themselves eating such products in the future, 21 % might buy it and 21 % would not buy it.
4.2.4. Usual snacks

For 60% of the subjects fruits constitute basic snacks. Only 20% ate candy and cookies and 20% ate other snacks like sandwiches or no snacks at all.

5. DISCUSSION

5.1. Blood glucose levels

Since 21% of the NF curves and 36% of both placebo- and the no-NF curves showed the highest peaks of blood glucose levels, no clear differences were found. This may mean that placebo and the NF-containing product lead to glucose maxima in blood to the same extent. This might be a result of the high level of carbohydrates that undergoes hydrolysis, such as sucrose. Since all sucrose in the chocolate mass of test products was replaced with NC, which in turn is hydrolyzed slower than sucrose, the glycemic load of the test products should be significantly lower with lower maxima than that of placebo. However, the results do not seem to agree with this statement. The test products contain approximately 4.3 g NC/30 finished product, while Cloetta’s chocolate product contains approximately 9.3 g sucrose/30 g finished product. This means that placebo contains more than twice as much sugar than the test products, which should strengthen the reliability of the statement. When focused on the kind of carbohydrates that digest relatively quick, it is even found that the content of mono- and disaccharides in placebo is 10 times higher, ca 16 g compared with the test products, ca 1.6 g. Even the dietary fiber found only in test products (1.6 g) is expected to strengthen the effect of NC by slowing down the rise of blood glucose.

93% of the placebo curves showed rapid fluctuations compared to 71% for the NF curves and 64% for the no-NF curves. These rapid fluctuations are not healthy, and Cloetta’s chocolate product seems to be the least healthy from this aspect. No curves obtained showed clear evenness, but 21% of the NF curves, 7% of the no-NF curves and barely one (7%) of all placebo curves show some kind of evenness compared to the remaining two curves for the same person. 21% is a low but a significantly higher share compared to 7% or no evenness, which gives a small indication that the NF-containing product may be relatively more healthy. However, more participants and more repeats of all measurements are needed to obtain more comparable results.

If we look at the mean curve instead, we see that placebo lead to the highest peak, which is possible since placebo had the highest value of sucrose. Next highest peak is generated by the NF-containing product, and finally the NF-lacking product. This can be logical since NF should lead to a more effective blood glucose response because it increases the secretion of GLP-1, which in turn stimulates the insulin secretion. Furthermore, the blood glucose levels generated by the NF-lacking product returned to baseline clearly earlier (after 70 min) compared to the NF-containing product (after 108 min). This result does not agree with the statement that the glucose levels generated by NF-containing product should return to baseline relatively earlier due to more effective insulin response.
The largest area under the curve was generated by placebo (34.76 mmol.min/L), followed by the NF-containing product (26.42 mmol.min/L) and finally the NF-lacking product (19.66 mmol.min/L). This means that placebo had the least capacity to control postprandial increase in blood glucose, and the NF-lacking product had the largest capacity to control the increase. It was expectable that placebo would have the least control on blood glucose response due to its high value of sucrose compared to the other two products. The result that was not expected was, on the other hand, that the NF-lacking product showed more control on blood glucose response than the NF-containing product, since NF was expected to show better control on the blood glucose response (GLP-1 → insulin↑ → more effective response).

The earlier mentioned conclusions alone should not necessarily be the answer, because several factors might have played a role in the results. Possible factors can be when the test subjects had lunch and what lunch contained, stress, worry, exercise or other individual factors like BMI. Subjects maybe did not follow the criteria given before the study start so they might have eaten a too late lunch with apportions of carbohydrates, fat and protein that might have disturbed the results. This might been an explanation since every subject in this study had different baseline levels at different study days. Subjects may even have exercised during the days they should not exercise on. This can lead to different insulin sensitivity, since it is proven that insulin sensitivity increases with regular physical activity (44). The substance studied within this context is malonyl CoA. High levels of this substance are associated with insulin resistance, inadequate use of blood sugar levels by the body cells. The levels of malonyl CoA have been shown to be reduced by regular exercise, partly because the substance is produced in smaller amounts and partly because it breaks down faster. This was observed in both type II diabetics and healthy persons, where the malonyl CoA levels were decreased in both groups. In healthy persons, insulin sensitivity did not increase though, but it increased with 77 % in diabetics. This was explained by suggesting that healthy persons had good insulin sensitivity from the beginning and they probably needed higher exercise intensity to show a difference (44).

During physical or emotional stress, the sympathetic nervous system is dominating. Energetic physical activity and rapid ATP production is a result that is favored by high sympathetic tone. Mixed emotions, such as fear, anger and embarrassment stimulates the sympathetic division. Release of hormones by the adrenal medullae starts a number of physical responses called “the fight-or-flight response”. The response leads to reactions, such as constriction of blood vessels that supply nonessential organs like the kidneys and gastrointestinal tract, inhibiting of other processes that are not needed for the stressful situation (e.g. muscular movements of the gastrointestinal tract and lowered or inhibited digestive secretion) and increased glycogenolysis in the liver (4). Increased release of glucose by the liver is induced to increase the energy supply to the cells, which leads to increased blood glucose level (4, 27). This may be an explanation for why the obtained blood glucose responses look like they do (4).

The shape and the differences between the subjects could also be related to the difference in BMI, physical status and variations in digestion. Another factor worth mentioning is that the first study day was less controlled than the other two days. This included disorder regarding the blood test points and the subjects might have been relatively more worried or uncomfortable. The last hour of the test period, the nurses had to buy more syringes so time was lost, which might has caused stress. Even during the blood sampling, “older” blood was mixed with fresh blood, which might have affected the measurement. Another factor worth mentioning is that the used instrument (Glucose 201) should be calibrated before starting the “real” measurements, e.g. by using a 50 g glucose solution as a placebo. Furthermore,
significantly more subjects and more repeats of the same measurements are needed to show differences or similarities.

5.2. Hunger levels

Regarding hunger levels, it is even more difficult to come to any relevant conclusions. The ideal or the expected results would show lowest and latest hunger levels after eating the NF-containing product, slightly higher and earlier hunger levels after the NF-lacking product and highest and earliest hunger levels after placebo. But this was not the case here. Instead, none felt hunger during the first half hour after NF-lacking product intake, compared with 6% after NF-containing product intake and 19% after placebo intake. It was remarkable that more subjects felt hunger after the NF-containing product compared to the NF-lacking product. Hunger levels during the following half hour was still higher for the PinnoThin™-containing product, 38%, compared to the NF-lacking product, 21%, and placebo, 31%. Most subjects left with no hunger after the NF-lacking product, followed by 37% after placebo and finally 25% after the NF-containing product. This might mean that the NF-containing product brings the highest satiety compared to the other products.

However, the information about hunger levels might not be exact, since the subjects were reminded now and then about writing down hunger levels and some of them might have forgotten to write down the time they felt hunger. Thus, information bias and low seriousness might be factors that could have affected the results. Even here, what and when lunch was and how much energy each individual requires play a role. This means that it is not appropriate to conclude that the results only depend on the nature of the products.

5.3. Taste opinion

The results of the taste opinion show that other tastes are more appreciated than the apricot taste in the test products. Since more subjects (31%) disliked the products than liked it (25%), while 44% gave the products a score of medium, other tastes should be considered in the future. According to the subjects, fruit tastes like orange, banana and berries are more favorable. Other non fruit related tastes were more chocolate taste, nougat, cappuccino and cola. However, the taste was not a key issue in this project due to lack of time. On the other hand, the focus was directed on the effects the products had on the body.

5.4. Usual snacks

Most subjects (60%) recorded that they usually ate fruits to snacks, and fewer subjects (20%) ate candy and cookies. In what extent the subjects were true about that information is unknown, because bad conscience could be a factor that makes people not willing to tell that
they eat “too much candy” or “too little fruits”. This situation is imaginable especially in a situation like this, where focus was directed on healthy products.

Despite the test opinion, the market future for the products looks bright. More than half of the subjects, 57% definitely could imagine themselves eating such products (test product) in the future. 21% could possibly buy it, and just as many, 21%, would not buy it at all. Only two subjects gave reasons for why they would not buy it. One reason was because they were used to eat fruits, and the other reason was because it had to be more satiating to be a snack. The latter point of view should be considered in future research.

5.5. Summary

In summary, the results of the individual blood glucose curves show an indication that the test products are relatively healthier, since Cloetta’s chocolate product gave the highest level of fluctuations and hardly any even blood sugar curves. However, this indication is very weak since there were no clear differences between the single blood glucose maxima. Even the mean curves showed an indication that Cloetta’s chocolate product was less healthy, since it lead to the highest blood glucose peak and the largest iAUC. However, whether or not the subjects followed the criteria regarding the exercising and lunch time is unknown. More subjects and more repeats of same measurements are needed to be included in further studies to obtain more stable and comparable results. Also recommended is the use of a standard solution (e.g. a solution with known glucose concentration) to calibrate the measurement instrument.

The hunger levels did not follow the expected trend, since the NF-lacking product seemed to satiate most subjects while the NF-containing product satiated the least number of subjects. A number of biases could have played a role in the results thought, for example when and what lunch was, the individual energy requirement and how serious and focused the subjects were about reporting the hunger levels. Thus, recommendations for further studies would be to once more emphasize for new subjects the importance of being focused and serious. Maybe the age limit of new subjects should be changed to 20 instead of 18.

There are some recommendations that can be made for future development of the test products. A bigger sized product should be made, because bigger size satiates more. Even the NF value increases by increased product size. 1.5 g of NF might have been too low to show any effect on satiety, according to the results. The recommended amount of NF is 3 g to reduce snacking, which should be followed in further development of the products. The fact that the after-taste of the products was sharp, this should be of consideration by choosing other filling alternatives. More favorable fruits were orange, banana and berries, especially strawberry. The sweetness of the chosen fruit should also be considered since sweet fruits might contain high quantities of sucrose and other mono-and disaccharides. Even other filling alternatives can be used, for example the filling must not necessarily consist of 100% fruit concentrate. Instead, it can consist of fruit or mixed fruit extract, combined with NF, proteins and fiber. This might be advantageous since the NF and protein value increases and the level of fruit sugar can be reduced. NC can be added to the filling, which should give healthier blood sugar response. Fruits rich in antioxidants are thus preferable if fruit extract is used. Such combination will for sure even reduce the sharpness of the taste and the after-taste and
might enhance the chocolate taste. To increase the amount of NF, R7 (fat) can be replaced with NF.
6. CONCLUSION

The following conclusions are obtained from this project:

- The idea of making a healthy and satiating between meal snack seems to be successful, since the demand of such products increases. Thus, further studies to develop the products made in this study are recommended.

- Different filling can be used in further research. It might be consisting of fruit or mixed fruit extract, NF, proteins, fibre and isomaltulose. More fat from the chocolate mass could be replaced with NF, up to 3 g per 30 gram of product as recommended by the NF producer.

- FRP should be used in further studies, since its effect on lowering the fat content is now proved (p 3.2.3.).

- More cocoa powder can be added to enhance the chocolate taste in the final product.

- More subjects and higher minimum age like 20 should be included in further studies. Emphasis of the importance of following the criteria and high degree of seriousness about giving right hunger levels should be enhanced.

- More repeats of the blood glucose measurement should be done to obtain more comparable and exact results.

- It is important to make sure that all instruments needed in the study, for example enough syringes, are available in the study days. Sometimes blood clots at the syringe opening, which leads to disturbing the flow of fresh blood or any blood at all through the syringe.

- It is recommended to use a glucose solution or some other carbohydrate source as a placebo before starting the measurements of the “real” products, to make sure that the used instrument is working normally and the test subjects have a normal blood glucose response.
7. ACKNOWLEDGMENT

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42. X.

43. X

44. Karolinska Institutet,

Hälsodeklaration - *Ett mellanmål: En nyttig och mättande stycksak*

Namn:  
Personnr:  
Postadress:  
Telefon/mobil:  
Datum:  

Vikt:  
Längd:  

1. Har du någon hjärtssjukdom/kärlsjukdom?  
   □ Ja □ Nej  

2. Har du blodbrist?  
   □ Ja □ Nej  

3. Har du järnbrist?  
   □ Ja □ Nej  

4. Har du sjukdom i mage, tarmar, pankreas eller annat bukorgan?  
   □ Ja □ Nej  

5. Har du diabetes?  
   □ Ja □ Nej  

6. Har du blodcancer/leukemi?  
   □ Ja □ Nej  

7. Har du hepatit A/B?  
   □ Ja □ Nej  

8. Har du någon leversjukdom?  
   □ Ja □ Nej  

9. Har du högt/lågt blodtryck?  
   □ Högt □ Lågt □ Normalt
APPENDIX 1 [2/3]

10. Röker/snusar du?
   □ Röker □ Snusar □ Ingetdera

11. Har du eller har haft ätstörningar?
   □ Ja □ Nej

12. Har du ordinerats mediciner för långtidsbruk?
   □ Ja □ Nej
   Om ja, ange vilka:

13. Äter du all sorts mat?
   □ Ja □ Nej
   Om nej, ange varför (t.ex. pga. glutenallergi, laktosintolerans mm):

14. Anser du dig själv som helt frisk?
   □ Ja □ Nej

15. Hur ofta tränar/motionerar du?
   □ Mer än 4 gånger/vecka □ 3 gånger/vecka □ Någon gång/vecka
   □ Några gånger per månad □ Inte alls

16. Vad tränar du?
   □ Styrketräning □ Motionsträning (springa, aerobics, dans mm) □ Yoga/liknande
   □ Annat, ange vad:

NÄSTA SIDA INNEHÅLLER FRÅGOR OM CHOKLAD!
APPENDIX 1 [3/3]

1. Hur ofta äter du choklad/kexchoklad?
   - En eller fler gånger/dag
   - Några gånger/vecka
   - Några gånger/månad
   - Sällan, några gånger/år
   - Inte alls

2. Angående mörk och ljus choklad, vad föredrar du?
   - Mörk choklad
   - Ljus choklad (mjölkchoklad)
   - Det kvittar
   - Ingetdera

3. Vad köper du helst?
   - Choklad utan kex
   - Kexchoklad

4. Kan du tänka dig köpa en kexchoklad med mindre fett?
   - Ja
   - Nej
   Om Nej, varför inte? :

5. Vad hade du köpt för produkt?
   - Hellre en produkt med mer mörk choklad, även om det innebär mer fett
   - Hellre en produkt med mindre fett (mjölkchoklad)

6. Vad vet du om sockerersättare? (OBS. sockerersättare ≠ sötningsmedel)
   - Farliga
   - Bättre alternativ än vanligt socker
   - Jag vet inte riktigt vad det innebär
   Om Farliga/bättre än socker, varför?
   …………………………………………………………………………………………………

7. Kan du tänka dig köpa en kexchoklad med ett annat socker än det vanliga men som inte belastar kroppens insulinsvar lika mycket och som inte ger karies?
   - Ja
   - Nej
   Om Nej, varför inte? ………………………………………………………………………

   - Socker
   - Fett

   Tack!
Health Declaration – A snack: A healthy and satiating snack

Name:  
Personal Identity Number:  
Postal address:  
Phone/mobile:  
Date:  

Weight:  
Height:  

17. Do you have any cardiovascular disease?  
☐ Yes  ☐ No  

18. Do you have blood deficit?  
☐ Yes  ☐ No  

19. Do you have iron deficit?  
☐ Yes  ☐ No  

20. Do you have disease in stomach, intestines, pancreases or any other abdomen organ?  
☐ Yes  ☐ No  

21. Do you have diabetes?  
☐ Yes  ☐ No  

22. Do you have blood cancer /leukemia?  
☐ Yes  ☐ No  

23. Do you have hepatitis A/B?  
☐ Yes  ☐ No  

24. Do you have any liver disease?  
☐ Yes  ☐ No  

25. Do you have high/low blood pressure?  
☐ High  ☐ Low  ☐ Normal
APPENDIX 1 [2/3]

26. Do you smoke/snuff?
☐ Smoke  ☐ Snuff  ☐ Neither, nor

27. Do you have or have had eating disorders?
☐ Yes  ☐ No

28. Have you been prescribed medication for long time use?
☐ Yes  ☐ No
If yes, which?:

29. Do you eat all food sorts?
☐ Yes  ☐ No
If no, why (e.g. due to gluten allergy, lactose intolerance etc.):

30. Do you believe you are a completely healthy?
☐ Yes  ☐ No

31. How often do you exercise?
☐ More than 4 times a week  ☐ 3 times a week  ☐ One time a week
☐ A few times/month  ☐ Never

32. What sort of exercise do you do?
☐ Fitness training  ☐ Keep-fit exercise (jogging, aerobics, dance mm)  ☐ Yoga/
something like that
☐ Other, what?:

NEXT PAGE INCLUDES QUESTIONS ABOUT CHOCOLATE!
9. How often do you eat chocolate/ wafer chocolate?

☐ One or several times/ day  ☐ Several times/month  ☐ A few times/month

☐ Seldom, a few times/year  ☐ Never

10. Regarding dark and light chocolate, which do you prefer?

☐ Dark chocolate  ☐ Light chocolate (milk chocolate)  ☐ Doesn’t matter

☐ neither, nor

11. What do you rather buy?

☐ Chocolate without wafer  ☐ Wafer chocolate

12. Would you buy a wafer chocolate with less fat?

☐ Yes  ☐ No

If no, why not? :

13. What would you buy?

☐ Rather a product with dark chocolate, even if it meant more fat

☐ Rather a product with less fat (milk chocolate)

14. What do you know about sugar replacers? (PS. Sugar replacers ≠ sweetener)

☐ Dangerous  ☐ Better alternative than common sugar  ☐ I don’t really know what it is

If dangerous/ better than common sugar, why?

…………………………………………………………………………………………………………………………

15. Would you buy a wafer chocolate with another sugar than common sugar, which does not load the body’s insulin response as much as common sugar and does not develop tooth decay?

☐ Yes  ☐ No

If no, why not? ……………………………………………………………………………

16. Which do you think gives more calories? PS. Mark the answer that you thin/now is correct!

☐ Sugar  ☐ Fat