LIST OF PUBLICATIONS

The PhD thesis includes the following three original papers, which will be referred to as:

Paper 1:

Kia Halschou Hansen, Klaus Bukhave & Jens Rikardt Andersen. **Intestinal disaccharidase activity and uptake of glucose from sucrose**, in A Lazinica, S Chackrewarthy (eds): Glucose Tolerance, InTech, 2012: 149-62
ISBN 978-953-51-0891-7.

Paper 2:

Kia Halschou-Jensen, Knud Erik Bach Knudsen, Søren Nielsen, Klaus Bukhave & Jens Rikardt Andersen**. Effect of L-arabinose on glucose uptake from mixed meals.**

Status: Manuscript ready for submission, Br J Nutr.

Paper 3:

Inger Krog-Mikkelsen, Sesilje Bondo Petersen, Kia Halschou-Jensen, Ole Hels, Jens Juul Holst, Jens Rikardt Andersen& Klaus Bukhave: **Effects of xylose on intestinal sucrase activity, in vitro and in humans.**

Status: Manuscript submitted, Am J ClinNutr

SUMMARY IN ENGLISH

The current health problems regarding the obesity epidemic, development of type 2 diabetes mellitus (T2D) and cardiovascular disease are a major challenge for healthcare systems worldwide.

No simple or unique cure has been documented to prevent or treat this major health problem regarding T2D and the risk factors related to this disease. There is therefore an immediate need for prevention methods and effective approaches to overcome these serious health issues.
It will need a combination of several interventions. The treatment of T2D today is primarily lifestyle changes like increased physical activity and change of diet, which corresponds to the treatment of insulin resistance, IGT and obesity. Secondly, a variety of medicine is used.

Within nutrition, one of the research areas is preventive or therapeutic aims against development of T2D. A better glycaemic control is one preventive target and furthermore it seems to be able to delay the incidence of T2D.Prandial regulation of glucose is a complex process and there are several methods to assess glycaemic control and thereby affect the blood glucose concentration. The prandial glucose regulation depends on factors including physical activity, the nature of ingested food, gastric emptying, intraluminal glucose concentration, and enzymatic activity in the brush border.

The focus in this review is on evidence provided by *in vitro* studies*,* animal models and human studies on L-arabinose, D-xylose and polyphenols. The focus is on their effects on carbohydrate- ingesting enzymes activity *in vitro* and possible effects on human postprandial blood response.

In **paper 1** the effects of sugar beet polyphenols from molasses and the potential inhibition of sucrase activity *in vitro,* was investigated. Two different polyphenol-rich fractions from chromatographic separation of molasses from sugar beets and pure ferulic acid were tested. We found no effects of the two fractions of molasses. The pure ferulic acid indicated an inhibition of sucrase *in vitro*.

Both *in vitro* and in *vivo studies* have investigated the effects of L-arabinose and D-xylose on carbohydrate digestive enzymes.

In **paper 3**, D-xylose and L-arabinose was investigated *in vitro* and *in vivo*. This study found that D-xylose and L-arabinose inhibit both sucrase and maltase when tested in a Caco-2 cell model. In addition, 13 healthy subjects completed a randomized double-blinded cross-over study with sucrose drinks supplemented with 4, 8 w/w% xylose or 8 w/w% L-arabinose. This showed that supplementa-

tion of 8% D-xylose and L-arabinose compared to pure sucrose produced a decline in blood glucose peak, as well as a decreased and delayed insulin peak.

These results from a sucrose drink added L-arabinose and D-xylose constituted the basis for the further investigations of L-arabinose. However, the use of higher dietary doses of sucrose would be unfeasible in terms of palatability in the human population.

In **paper 2,** the purpose was to investigate if the positive effects of L-arabinose added to a sugar drink could be reproduced in a mixed meal containing sucrose and/or starch. Furthermore the consistencies of the ingested meals and the possible effect on gastric emptying and thereby postprandial blood concentrations of glucose, insulin and C-peptide, were investigated.

In conclusion, this PhD thesis found no evidence that L-arabinose affects post prandial blood glucose, insulin and C-peptide when mixed in a meal. This might be due to the difference in gastric emptying rate between the fluid and solid meals, but the conclusion is associated with certain reservations regarding sample size (n=6) in the study and the method for measuring gastric emptying.
Furthermore, the fluid maltose drink could not validate the *in vitro* studies on maltase activity.
The overall concluding perspective must be that L-arabinose has the greatest potential to effect glucose and insulin secretion when added to a sucrose drink.

DANSK SAMMENDRAG (DANISH SUMMARY)

Den nuværende fedme epidemi, udvikling af Type 2 diabetes (T2D) og hjertekarsygdomme giver på verdensplanstore sundhedsmæssige udfordringer.

Der er på nuværende tidspunkt ikke påvist nogen simpel kur, forebyggelse eller behandling af T2D og de risikofaktorer der er relateret til sygdommen.

Det kræver en kombination af flere interventioner for at behandle og/eller forebygge T2D. I dag er den primære behandling livstilsændringer i form af øget fysisk aktivitet og kostomlægning, hvilket stemmer overens med behandlingen af insulinresistens, IGT og fedme. Sekundært bliver farmakologisk behandling anvendt i de situationer hvor livsstilændringer ikke er tilstrækkeligt.

Et af forskningsområderne inden for ernæring er forebyggelse og behandling af T2D. Her er optimeret glykæmisk kontrol et af de forebyggende tiltag der kan være med til at forsinke incidensen af T2D.

Den postprandiale regulering af glukose er en kompleks proces, og der er flere metoder hvorpå den glykæmiske kontrol kan påvirkes. Den postprandiale regulering af glukose afhænger af faktorer som fysisk aktivitet, fødeindtag, mavetømningshastighed, den intraluminale glukose koncentration og aktiviteten af fordøjelsesenzymerne i brush border membranen.

I denne PhD afhandling, fokuseres der på enzymaktiviteten i brush border membranen og evidens fra *in vitro* studier, dyrestudier og1 humane studier omhandlende L-arabinose, D-xylose og polyphenolers effekt på de kulhydrat- nedbrydende enzymer, og derved mulige effekt på det postprandiale respons.

Effekten af sukkerroe melasse på sukraseaktiviteten blev undersøgt *in vitro* og præsenteret i **paper 1.** To melassefraktioner fra sukkerroer, indeholdende polyphenoler samt ren ferulasyre blev testet. Vi fandt ingen inhiberende effekt af de to melassefraktioner. Den rene ferulasyre indikerede en hæmmende effekt af sukrase *in vitro.*

D-xylose og L-arabinose blev undersøgt *in vitro* og *in vivo*i **paper 3**. Dette studie fandt at D-xylose og L-arabinose hæmmer både sukrase og maltase i caco-2 celle homogenat. Yderligere, blev 13 raske forsøgspersoner randomiseret til et dobbeltblindet cross-over studie. Her indtog de en sukrose drik (75 g sukrose i 300 ml vand) tilsat 4 og 8 vægtprocent D-xylose og 8 vægtprocent L-arabinose. Resultaterne viste et nedsat glukose peak så vel som nedsat og forsinket insulin peak.

Disse resultater fra sukroseholdige drikke tilsat L-arabinose og D-xylose gav anledning til videre undersøgelser af L-arabinose. Dog er så høje doser af sukrose ikke klinisk relevant og praktiske formål uspiseligt på grund af smagen, hvorfor der i de videre undersøgelser blev brugt L-arabinose tilsat fast føde indeholdende normaliserede mængder af sukrose.

Formålet i **paper 2** var at undersøge om L-arabinose, og de positive effekter fundet i sukkerholdige drikke, er mulige at reproducere ved at tilsætte L-arabinose i et sammensat måltid indeholdende sukrose og/eller stivelse. Derudover blev konsistensen af måltidet og den mulige effekt på mavetømningshastigheden og dermed den postprandiale koncentration af glukose, insulin og C-peptid, undersøgt.

Der blev ikke fundet signifikante effekter på niveauet af postprandial glukose, insulin og C-peptid, når L-arabinose blev tilsat et sammensat måltid. Årsagen hertil kan ligge i forskellen på mavetømningshastigheden efter indtag af flydende og fast føde. Dog er denne antagelse forbundet med flere usikkerheder såsom stikprøvestørrelsen (n=6) og selve metoden til måling af tømningshastigheden.

Konklusionen og det videre perspektiv må derfor være, at L-arabinose har det største potentiale for at påvirke det postprandiale glukose respons og dermed insulinsekretionen når det bliver tilsat en sukroserig drik.

AIMS AND OBJECTIVES

Postprandial hyperglycaemia is now established as an independent risk factor for the development of at least macro vascular complications in diabetes mellitus (1). One of many ways to decrease this postprandial hyperglycaemia is by modulating the absorption of glucose from digested carbohydrates. The main favourable consequence of the lowering of postprandial glucose concentrations in the blood is most likely a decrease in the production of insulin, meaning that the same amount of glucose can be metabolized by a smaller amount of insulin.

Based on studies with L-arabinose and D-xylose, it is evident that these specific naturally occurring pentoses hold the potential to inhibit carbohydrate digestive enzymes and thereby reduce postprandial blood glucose levels, as well as insulin- and C-peptide production when added to sugar-containing drinks. However, ingestion of drinks with high content of sugar is rather unusual for diabetic patients, but probably very frequent for persons at high risk of Diabetes Mellitus, i.e. persons/patients with insulin resistance.

Besides pentose, polyphenols have also been a candidate for positive effects.

For public health purposes, the main effects would be expected if the blood glucose-lowering components could be incorporated in the usual and recommended diet for most of the population. Another practical aspect is that adding a component to sugar has much less effect than addition to our main glucose-supplying carbohydrate; starch.

Accordingly, the primary aim of this PhD thesis was to examine the potential of the pentose L-arabinose added in a mixed meal to inhibit carbohydrate digestive enzymes and the relation to postprandial blood glucose, insulin and C-peptide, as it is a widely accepted experience that it is more difficult to decrease the postprandial blood glucose than the fasting concentrations.

The secondary aims were to search for similar effects in polyphenols from molasses, which is a biproduct in the production of sucrose from sugar beets, and to look for effects on the handling of starch/maltose in the small bowel, *in-vitro* and *in-vivo*.

The hypothesis in the dissertation:

***In vitro* studies with maltase:**

Does L-arabinose and D-xylose inhibit maltase activity *in vitro* with maltose as a substrate? The purpose was to investigate if L-arabinose and D-xylose additionally could have an inhibiting effect on starch digestion similar to the one shown in sucrose digestion.

***In vitro* studies with polyphenols:**

Do sugar beet polyphenols from molasses inhibit sucrase activity *in vitro*?

Intervention study 1:

Does L-arabinose added to a solid, mixed meal inhibit sucrase and maltase activity in humans judged from postprandial plasma glucose, insulin and C-peptide? The purpose was to investigate if the positive effects of L-arabinose added to a sugar drink could be reproduced in a mixed meal containing sucrose and/or starch

Intervention study 2:

Does the consistence of the ingested meal affect gastric emptying and thereby postprandial blood concentrations of glucose, insulin and C-peptide, and does an increase in arabinose dose give better results?

CONTENTS

REVIEW: *Inhibition of intestinal disaccharidase activity by pentoses.*
Effects on glucose uptake from starch and disaccharides and insulin secretion in man

1 INTRODUCTION

The current health problems regarding the obesity epidemic, development of type 2 diabetes mellitus (T2D) and cardiovascular disease are a major challenge for the healthcare systems worldwide. It has been estimated by the International Diabetes Federation that 366 million people worldwide had diabetes mellitus in 2011 and by 2030 this will have risen to 552 million (2). A total of 306,638 persons were diagnosed with diabetes in 2011 In Denmark; a twofold increase in ten years (3). T2D accounted for around 80%, and 200,000 persons were undiagnosed with T2D. A total of 750,000 Danes had prediabetes in 2010 and it is expected that 30 - 40% of these will develop T2D within 3-5 years {Rasmussen, 2008 286 /id}.

The fight against this growing health problem requires the academic sector to work innovatively to improve and develop tools for preventive or therapeutic aims. Within nutrition, one of the research areas is better glycaemic control. The glucose metabolism is in focus, as even a slight increase in the normal postprandial glucose response after food intake correlates with the mechanism behind developing T2D (5;6) and actually poses a risk for developing insulin resistance (7). Furthermore, several studies indicate that the development of insulin resistance is a major risk factor for developing T2D, hypertension and cardiovascular diseases (8;9).

With insulin resistance being a key factor in T2D, the same risk factors for T2D generally apply for insulin resistance. The 750,000 prediabetic persons with impaired glucose tolerance and insulin resistance become an interesting group for a health promoting intervention to prevent the development of T2D and the risk of early deathfrom CVD.

A diet in which fruit, non-starch vegetables, and dairy products has been emphasized as being useful for people with T2D patients compared to high starch meals or typical “American” western diets (10) which leads to an interest in modifying the intake or digestion of starch and sugar. Altering the intake of sugar and starch would probably be a challenge in the industrialized countries, such as Denmark, where the overall intake of starch and sugar are high (11). Nutritional scientists and food industry have been working with the nature of ingested food and possible carbohydrate digestive inhibitors. Pentoses and polyphenols are among of the inhibitor candidates. The pentose types L-arabinose and D-xylose are poorly-absorbed, readily-available sweet-tasting pentose sugars. They have both showed an inhibiting effect on sucrase activity *in vitro* (12-14) and studies with different polyphenols have

reported similar effects. The proper application of pentoses or polyphenolic compounds in different sucrose-based products may lessen the undesired effects of sucrose and starch.

The focus in this review is on evidence provided by *in vitro* studies*,* animal models and human studies on L-arabinose, D-xylose and polyphenols. The focus is on their effects on carbohydrate ingesting enzymes *in vitro* and possible effects on human postprandial blood response. The review has four aims:

1) Investigation of the effect of L-arabinose and D-xylose on maltase activity *in vitro*.

2) Investigation of sugar beet molasses or fractions of sugar beet molasses, containing a variety of different polyphenols, for inhibiting effect on brush border sucrase and maltase *in vitro*

3) Investigation of L-arabinose added to a solid mixed meal and the inhibition of sucrase and maltase activity evaluated from blood glucose, insulin and C-peptide after ingestion.

4) Investigation of gastric emptying and the possible effects on postprandial blood parameters after ingesting L-arabinose in mixed meals with different structures.