General information	
PPP-number	AF 16127
Title	Assessment of the glycaemic effects of infant nutrition at weaning on long term metabolic and gastrointestinal health in the Göttingen Minipig model.
Theme	Voeding & Gezondheid (Nutrition and Health)
Implementing institute	Wageningen Livestock Research (WLR) and Wageningen BioVeterinary Research (WBVR)
Project leader research (name + e-mail address)	Sietse Jan Koopmans, sietsejan.koopmans@wur.nl
Coordinator (on behalf of private partners)	José Manuel Ramos Nieves, Nestlé Research Center, Lausanne, Switzerland
Project-website address	
Start date	01-01-2017
Final date	31-12-2019

Approval by the coordinator of the consortium The final report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the final report.	
Assessment of the report by the coordinator on behalf of the consortium:	x Approved ☐ Not approved
Additional comments concerning the final report:	

Consortium	
Mention any changes in the composition of the project partners:	

Summary of the project	
Problem definition	Introduction and aim: Increasing evidence suggests that early life environment can influence health and disease later in life. Optimization of nutrition during infancy could decrease the risk of non-communicable diseases thus having an important potential impact on public health and economy. In this study, we hypothesize that diets promoting a high postprandial glucose response during the weaning period have a detrimental effect on metabolic health later in life, by affecting organ function and development. The alterations will result in the programming of metabolic disease. We will approach this question by feeding experimental diets eliciting high and low postprandial glucose responses to weaned Göttingen Minipigs for 9 weeks and monitoring their metabolic health until adulthood. What is the situation?

	In adults, frequent consumption of high-postprandial- glucose-response diets is associated to greater risk of type 2 diabetes and cardiovascular disease. Up to now, the effect of carbohydrate choices in infants is not known. Several organs and systems are still in development at this stage and thus may be sensitive to be programmed.
Project goals	How does the project address this situation/question? We will approach this question by feeding experimental diets eliciting high and low postprandial glucose response to weaned Göttingen Minipigs for 9 weeks and monitoring their metabolic health along the experiment and until adulthood. Göttingen Minipigs are the model of choice because of their similarities to the human infant in terms of digestive tract, and because they develop features of metabolic syndrome. Newly born minipigs reach adulthood within 6 months of age.

Results	
Planned results in the original project plan	What are the results of the project? Generation of pre-clinical knowledge on the mechanisms by which high and low glycemic infant nutrition may affect health in later life.
Achieved results	This project includes three different studies: 1) Diet validation. Objective: To determine the postprandial glucose and insulin responses after consumption of experimental diets in 5-6 weeks old male and female Göttingen Minipigs. Method and results: six experimental diets were formulated based on ingredients known to affect postprandial blood glucose and insulin concentrations. Various slow and fast digestible carbohydrates were used together with proteins and amino acids known to affect insulin secretion. These 6 experimental diets were screened for postprandial glucose and insulin responses in 6 week old minipigs. Out of these 6 experimental diets, two contrasting glycemic-insulinemic diets were chosen for the remaining of the study. The chosen low and high glycemic diets differed more than 5-fold in their postprandial blood glucose ($p<0.001$) and insulin responses (incremental area under the curve) in the 3 hour post-prandial time period. 2) Effect of age on post-prandial glucose and insulin response. Objective: To determine the postprandial response after consumption of the 2 chosen low and high glycemic diets in female Göttingen Minipigs of 2 months of age and compare that to the response in the same Göttingen Minipigs at the age of 10 months. Method and results: the female minipigs were kept in group-housing and fed a normal SDS-minipig diet from 2 till 10 months of age. At 2 and 10 months of age, the 2 selected diets from study 1 were tested for postprandial glycemic and insulinemic responses. At 2 months of age, the low and high glycemic diets differed ~5-fold ($p<0.001$) in their postprandial blood glucose response (area under the curve). The low and high glycemic diets differed ~3-fold ($p<0.001$) in their postprandial plasma insulin response (area under the curve). At 10 months of age the low and high glycemic diets differed ~7-fold ($p<0.001$) in their postprandial blood glucose response (area under the curve). The low and high glycemic diets differed ~5-fold ($p<0.001$) in

	their postprandial plasma insulin response (area under the curve). We conclude that low and high glycemic diets, when tested in the infant or adult phenotype, show pronounced differences in their postprandial glycemic and insulinemic responses irrespective of age, yet a greater contrast in postprandial blood glucose and plasma insulin responses in the adult phenotype. Time to maximum glycemia was later in young Göttingen Minipigs. Insulinemic response to high glycemic diet was greater in 10 month old compared to 2 month old Göttingen Minipigs. 3) Long-term study. Duration: 12 months Objective: To determine the short- (3 months), medium- (6 months) and long-term (9 and 12 months) effects of the experimental low and high glycemic weaning diets on cardio-metabolic health of female Göttingen Minipigs. Method and results: Fourty-eight 3-week-old weaned female minipigs were balanced on body weights and litter to receive either the low or high glycemic diet for a period of 9 weeks (Stage I). Male suckling and male post-weaning minipigs (fed the low or high glycemic diet for a period of 2 weeks) were euthanized and blood and digesta samples were collected for baseline purposes. At the end of the 9-week period, the female minipigs were studied for insulin sensitivity (hyperinsulinemic clamp), insulin secretion (hyperglycemic/arginine clamp), blood pressure and heart rate. After metabolic and cardiovascular testing, piglets were euthanized for portal and peripheral blood collection, digesta collection and tissue/organ collection for further detailed information on the phenotype and possible programming of the minipigs. From 3 months to 6 months of age (Stage II), the remaining minipigs (n=32) were offered a controlled and fixed amount of regular chow diet in proportion to their body weight to achieve an average body weight gain of 1 kg per week. Minipigs were studied at 6 months of age using the same techniques as used for the Stage I piglets. From 6 months to 12 months of age (stage III), the remaining minipigs (n=16) were offered a controlled and fixed amount of obesogenic Western diet until the end of the experiment at 12 months of age to achieve an average body weight gain of 2 kg per week. Minipigs were studied at 12 months of age using the same techniques as used for the Stage I and II piglets. Analyses of the results indicate that: 1) Early life glycemic nutrition affects insulin secretion in early and later life when fed a normal chow diet. Early life glycemic nutrition does not affect insulin secretion in later life when fed an obesogenic diet. 2) Early life glycemic nutrition affects blood pressure in later life when fed a normal chow diet. However, a very obese phenotype renders both groups hypertensive after consumption of the obesogenic diet for 6 months. 3) Early life glycemic nutrition affects baseline plasma glucose and free fatty acids and tends to increase baseline plasma insulin concentrations whereas baseline cholesterol, LDL, HDL, and triglycerides are not affected in later life when fed a normal chow diet. Early life glycemic nutrition affects baseline plasma FFA whereas baseline plasma glucose, insulin, cholesterol, LDL, HDL, and triglycerides are not affected in later life when fed an obesogenic diet. 4) Early life glycemic nutrition does not affect insulin sensitivity but influences body composition towards an increased muscle mass and muscle to fat ratio in later life when fed a normal chow diet.

	Early life glycemic nutrition does not affect insulin sensitivity nor early atherosclerosis but tends to increase body fat content in later life when fed an obesogenic diet. Overall this study shows that early life glycemic nutrition has a programming effect on cardiometabolic health in later life. High glycemic infant nutrition increases the activity the insulin-axis in adolescents resulting in increased muscle anabolism at the expense of an elevation in systolic blood pressure. Later on, in young adults, when exposed to an obesogenic Western diet, high glycemic infant nutrition predisposes to increased fat accretion and adiposity. Conclusion: Increasing the activity of the insulin axis may promote and /or accelerate (endogenous) development: increased muscle anabolism in young growing infants-adolescents and a switch to more fat deposition at the end of the growth curve when adult. Possible implication: high glycemic infant nutrition may be beneficial in infants showing impaired body development, whereas low glycemic infant nutrition may be beneficial in infants showing normal or excessive body development.
Explanation of changes relative to the project plan	More focus was on cardiometabolic health instead of metabolic health per se. Blood pressure and early atherosclerosis were investigated in more detail whereas research on gastrointestinal microbiota still needs to be conducted. However, digesta samples at various locations along the gastrointestinal tract have been collected from all Göttingen Minipigs and stored at -80 C for later analyses.

What was the added value created by the project for:	
Participating "Knowledge Institutes" (scientific, new technologies, collaboration)	This project created an inspiring platform to implement newtechnology with existing knowledge and skills on multidisciplinary pre-clinical and translational research on nutrition, metabolism, cardiology, endocrinology and health in a Göttingen Minipig model for human developmental (patho)physiology. The collaboration and interaction between academia (WUR being WLR and WBVR together with Erasmus Medical Center Rotterdam), producers of food ingredients and food end-products, and the breeder of Göttingen Minipigs proved to be very effective in learning and implementing new skills and techniques for preclinical research on human nutrition.
Participating private partners (practical application of the results, within which period of time?)	Given the low level of available information on the role that carbohydrate quality and quantity might play in infant development and programming of future health, these results are of capital importance to the industry. They offer initial evidence on the potential benefits of reformulation of infant products. Also, the data generated in these experiments opens new avenues of research to be expanded and further explored. For the breeder of pre-clinical large animal models for human translational research, the Göttingen Minipigs has been validated as relevant test model for human infant and adult nutrition.
Society (social, environment, economy)	This project will provide unique information on the role that carbohydrate quality and quantity might play in infant development, metabolism and programming of future health. The results will be communicated to a broad scientific audience and health-care professionals on infant nutrition, contributing to create awareness on the importance of carbohydrate choices

	during the complementary feeding period with potential beneficial societal impact.
Possibly other stakeholders (spin-offs)	

Follow-up	
Did the PPP result in one or more patents (first filings)?	
Are there any follow-up projects planned? If yes, explain. (Contract research resulting from this project, additional funding, or new PPP projects)	To be discussed

Deliverables/products during the entire course of the PPP (provide the titles and/or a brief description of the products/deliverables or a link to a website.
Scientific articles:
External reports: WUR report on: Early life glycemic nutrition and later life cardiometabolic health in Göttingen Minipigs (2020).
Articles in professional journals/magazines:
(Poster) presentations at workshops, seminars or symposia. Ramos Nieves J. Manuel, Koopmans Sietse-Jan, Mikkelsen Lars Friis, Theis Stephan, Castaneda Gutierrez Euridice. Diets inducing early life high-glycemic/insulinemic postprandial response program systolic arterial pressure. Abstract-Poster at the World Congress of the International Society for Developmental Origins of Health and Disease at Melbourne, Australia, Oct 2019.
TV/ radio / social media / newspaper:

Remaining deliverables (techniques, devices, methods, etc.):