

University of Groningen

In vivo estimation of gluconeogenesis

Chacko, Shaji Kurian

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:
2011

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Chacko, S. K. (2011). *In vivo estimation of gluconeogenesis*. [Thesis fully internal (DIV), University of Groningen]. [s.n.].

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Chapter 8

Summary, General Discussion and

Future Perspectives

A simple yet accurate and reproducible measurement technique is often a prerequisite for quality research without which no worthwhile finding is possible within a reasonable amount of time. The complexity, tediousness and high costs have limited the broad use of stable isotope methods to determine the quantitative *in vivo* estimates of gluconeogenesis in glucose metabolic research.

The primary objectives of this thesis were to develop a simple, accurate and reproducible method with high sample through-put to measure gluconeogenesis and further, to estimate *in vivo* rates of gluconeogenesis in different populations under various conditions applying the new and other methods to address various metabolic issues.

In **Chapter 2** we report a highly reproducible and simple/sensitive method requiring only small sample volumes, thus, providing a tool to study the details of glucose kinetics that can be used by many investigators, thereby forwarding research on glucose metabolism.

After the ingestion or infusion of deuterium oxide and equilibration of deuterium in the total body water pool, deuterium is incorporated into intermediary substrates along the glycolytic/gluconeogenic pathway. The isomerization of glyceraldehyde-3-phosphate to dihydroxyacetone phosphate by triose phosphate isomerase and a series of equilibration reactions between phosphoenolpyruvate and dihydroxyacetone phosphate is consistent with deuterium incorporation in C-1, 3, 4, 5 and 6 of glucose during the gluconeogenic process. The ^2H enrichment at C-2 is purported to be due to complete ^2H exchange with body water during the extensive glucose-6-phosphate to fructose-6-phosphate isomerization and therefore, does not specifically reflect the gluconeogenic process. Thus, the degree of deuterium labeling in plasma glucose C-1, 3, 4, 5 and 6 is a

measure of gluconeogenesis. Among the various GC-MS fragments of glucose derivatives considered, the m/z 170/169 fragment of the pentaacetate derivative was selected because of the presence of all the exchangeable hydrogen in C-1, 3, 4, 5 and 6 of glucose during the gluconeogenic process. We showed that this method is robust in that the enrichments measured using the m/z 170/169 fragment does not fluctuate over a wide abundance range.

Despite differences in substrate availability under conditions of overnight and extended fasting (66 h), and total parenteral nutrition providing glucose at high infusion rates (and various substrates at high concentration), the average enrichment method provided results comparable with the C-5 HMT method with a CV of <3%. Thus, the results obtained at high, intermediate and low fractional gluconeogenesis compared very well between our average enrichment method and the C-5 HMT method. Because it is very accurate and reproducible even when fractional gluconeogenesis is low, this new method can be potentially used in subjects receiving parenteral or enteral feedings and during insulin clamp studies.

The low tracer cost and simplicity of analysis make this method affordable and accessible to a wide number of investigators and it can be completed in a few hours. In addition, the small sample volume requirement makes the method applicable to studies in infants, children and small laboratory animals.

There are no published reports on total gluconeogenesis in very premature infants receiving routine total parenteral nutrition providing glucose at rates exceeding normal infant glucose turnover rate, which often results in hyperglycemia. In the study described

in **Chapter 3**, we determined whether gluconeogenesis is sustained in very premature infants receiving standard nutritional care, and if it correlates with glucose infusion rate and/or blood glucose concentration. To ascertain and to compare the accuracy of the measurements of gluconeogenesis under conditions of high exogenous glucose infusion rates, we measured gluconeogenesis applying both our new average deuterium enrichment method and the C-5 HMT method reported by Landau et al.

The study demonstrated that in very premature infants, gluconeogenesis is an ongoing process even when glucose is supplied at rates exceeding their normal glucose turnover as part of TPN. Further, gluconeogenesis accounts for the major part of residual glucose production and thus, the incomplete suppression of glucose production observed in preterm infants is primarily due to the contribution from gluconeogenesis. In very premature infants, gluconeogenesis was not affected by infusion rates of glucose, lipid and amino acids or blood glucose concentration, gestational age and birth weight.

Our studies showed that despite the infants' glucose and energy needs are supplied by the parenteral nutrition, gluconeogenesis was sustained indicating a lack of ability to regulate this process. During our investigations on factors affecting blood glucose concentration, we found that glucose appearance rate (i.e. glucose infusion + glucose production) and gestational age explained ~ 79% of the variation in blood glucose concentration in these infants. Additionally, the agreement between the estimates of gluconeogenesis obtained by the two methods applied in the present study demonstrated that the new approach compare very well with the published Landau method, thus, validating the new method even under conditions of low fractional gluconeogenesis.

The results from this study suggest that a potential strategy to prevent hyperglycemia without increasing the risk of hypoglycemia or insufficient energy intake would be to provide a TPN solution supplying glucose at a rate equivalent to the normal infant glucose production rate in addition to parenteral lipids and amino acids during the first days of life.

There are no reports on the hormonal regulation of gluconeogenesis in preterm infants. In the study reported in **Chapter 4**, we investigated potential factors regulating gluconeogenesis in Extremely Low Birth Weight (ELBW) infants receiving TPN. This was achieved by measuring gluconeogenesis during routine TPN providing glucose at high rates and also in response to reducing the glucose infusion to half normal newborn glucose turnover rate.

We evaluated the impact of the subsequent changes of insulin and glucose concentrations occurring in response to the change in the glucose infusion rate on gluconeogenesis. Lack of changes in gluconeogenesis clearly demonstrated that gluconeogenesis is not acutely affected by either insulin or glucose concentrations in ELBW infants receiving TPN. However, a strong relationship between the decreases in glucose and insulin concentrations between high and low glucose infusion periods demonstrated that these immature infants are capable of adjusting insulin in response to the lower glucose concentrations.

Insulin counterregulatory hormones, glucagon and cortisol, which primarily function during hypoglycemic conditions remained unchanged and this might be because of the normoglycemia maintained in the infants and availability of gluconeogenic

substrates via TPN. This is the first report in which both constituents of glucose production; gluconeogenesis and glycogenolysis are measured during routine TPN providing glucose at high and at reduced infusion rates (half the infant glucose turnover rate) in addition to parenteral lipids and amino acids.

In this study we demonstrated that gluconeogenesis is an ongoing process enabling preterm infants receiving TPN to remain normoglycemic even during a glucose supply corresponding to half normal turnover rate. This further supports our earlier report (Chapter 3) recommending that maintaining a glucose infusion rate equivalent to normal infant glucose turnover rates as part of total parenteral nutrition is a potential approach to prevent both hypo- and hyperglycemia and yet provide sufficient energy for growth in ELBW infants during their first days of life. The results also confirm our previous report showing that the glucose infusion rate is the primary factor that can be optimized to reduce the risk of hyperglycemia.

Additionally, our data demonstrated that despite substantially higher insulin concentrations during infusion of glucose at high rates, blood glucose concentrations were elevated. This indicates that the use of early insulin therapy might be a questionable approach to control glucose concentration in extremely low birth weight infants.

Further, similar protein turnover data during the two glucose infusion periods demonstrated that reduced supply of glucose as a part of TPN does not increase proteolysis in extremely low birth weight infants. This might imply that providing gluconeogenic substrates via TPN prevented a potential need of increased proteolysis to sustain gluconeogenesis to meet glucose demands even when the glucose supply is low.

Collectively, the data from chapters 3 and 4 indicates that supplying glucose at rates corresponding to normal infant glucose turnover rate in addition to glucose produced via gluconeogenesis (utilizing the parenteral lipid and amino acid substrates) is a potential strategy to prevent hypo/hyperglycemia in infants during their early days of life.

In **Chapter 5**, we established how lactating women manage their increased glucose demands during extended fasting periods without compromising the lactation significantly.

Our study in six healthy exclusively breastfeeding women and six non-lactating controls during a 42 h of fast, demonstrated that extra glucose demands of lactation during extended fasting are met by increased gluconeogenesis. After 42 h of fasting, we observed that milk production remained within the normal range with only 16% reduction in milk volume. Glucose, insulin and C-peptide concentrations decreased with the duration of fasting in both groups but were lower in lactating women. Glucagon, FFA and β -hydroxybutyrate concentrations increased with fasting time and were higher in lactating women during both fasting and re-feeding. Although gluconeogenesis was higher in lactating women when compared to non-lactating controls during 42h of fasting, glycogenolysis was not different.

Interestingly, we found that mammary hexoneogenesis remained unchanged throughout the entire duration of fasting despite increased risk of hypoglycemia in lactating women. Further, our data demonstrated that carbohydrate oxidation was lower and fat and protein oxidation higher in lactating women.

This study revealed that the extra glucose demands of lactation during the extended fasting were met by increasing gluconeogenesis. Nonetheless, lactating women are at risk for hypoglycemia beyond 30h of fasting.

Although reduced plasma ghrelin concentration has been suggested as a potential mechanism for the improvement of glucose metabolism in patients after bariatric surgery procedures, the association between the improvement of glucose metabolism and ghrelin concentration is not yet defined. In **Chapter 6** we report a study measuring glucose kinetics in ghrelin and growth hormone secretagogue receptor deficient mice models to assess the beneficial effects of the absence of ghrelin action on glucose metabolism. We demonstrated that ablation of ghrelin or its receptor resulted in increase of gluconeogenesis and glycogenolysis, following an 8 h of fasting. These data suggest that ghrelin plays a role in the regulation of gluconeogenesis and glycogenolysis, and that at least part of these effects occurs via the growth hormone secretagogue receptor.

The increased rate of gluconeogenesis and glycogenolysis might be because of the absence of the inhibiting effect of ghrelin on leptin. Leptin was previously demonstrated to enhance gluconeogenesis and diminish insulin secretion; however, antagonizing effects of ghrelin on leptin action have also been reported.

Significantly higher hepatic insulin sensitivity index and improved insulin resistance calculated by the HOMA-IR in ghrelin^{-/-} and Ghhr^{-/-} as compared to WT mice demonstrate increased insulin sensitivity in the absence of ghrelin action on the purported ghrelin receptor. Higher glucose clearance observed at lower insulin concentration during short term fast in ghrelin^{-/-} and Ghhr^{-/-} as compared to WT mice might suggest

that less insulin is required for peripheral glucose uptake in the absence of ghrelin or its receptor under normal physiologic conditions. During prolonged fasting (18 h), glucose clearance was reduced in ghrelin deficient mice.

Thus, our study demonstrates that gluconeogenesis and glycogenolysis are increased, and insulin sensitivity is improved by the ablation of ghrelin or its receptor in mice. Thus, reduced ghrelin might be an explanation for the improvement of glucose metabolism seen in patients after bariatric surgery procedures. In addition, the data from this study in transgenic mice models support a potential prospect of improving insulin sensitivity in the diabetic condition by the use of ghrelin receptor antagonists.

Finally in **Chapter 7**, we demonstrated a study technique that can be used to facilitate metabolic studies under real-life conditions without interfering with the normal activity of human subjects. We employed subcutaneous tracer infusion and “finger stick” blood sampling methods to validate this technique. The comparison of data from simultaneous use of intravenous and subcutaneous infusion using two glucose tracers ([1-¹³C]glucose and [6,6-²H₂]glucose) and corresponding venous and “finger stick” blood sampling demonstrated that subcutaneous infusion and “finger stick” blood sampling in humans is a potential alternative to measure appearance rate of glucose under real life circumstances.

As anticipated the intravenously infused tracer achieved near steady state faster than the subcutaneously infused tracer. However, near steady state was achieved by both infusion methods within a reasonable period of ~7 hours. The rate of decay was not

significantly different between the intravenous and subcutaneous tracer infusions regardless of the blood sampling site (venous vs. finger stick).

The rate of appearance using the [1-¹³C]glucose data was ~17% lower than for [6,6-²H₂]glucose. This difference is most likely a result of the recycling of ¹³C glucose into the circulation via the Cori cycle. The slight delay in decay of [1-¹³C]glucose compared with [6,6-²H₂]glucose observed may also be explained by the recycling of tracer via the Cori cycle and also by the delayed entry of subcutaneously infused tracer into the circulation via the lymphatic system. This study technique could be potentially utilized to execute large studies in a home setting, which would otherwise not be economically feasible to carry out in a clinical research setting.

Future perspectives

- There are no data on the effect of exogenous insulin (or early insulin therapy) on gluconeogenesis and glycogenolysis in preterm infants under routine total parenteral nutrition providing glucose at rates exceeding the normal infant glucose turn over. Studies addressing this issue are necessary to evaluate potential benefits of early insulin therapy. Simultaneous measurement of protein kinetics would provide an opportunity to assess potential benefits of early insulin therapy on protein metabolism in addition to glucose kinetics.
- Using the mice models to evaluate the utilization of ghrelin receptor antagonists to improve insulin sensitivity in diabetic state.

- Ghrelin infusion in ghrelin transgenic mice models should be tested to investigate whether ghrelin induces insulin resistance. Thus, the role of ghrelin in the improvement of insulin sensitivity can be further evaluated.
- Ghrelin infusion in growth hormone secretagogue receptor knockout mice should be performed to confirm the absence of any supplementary relevant receptors for the hormone ghrelin in mice (other than ghrelin's purported growth hormone secretagogue receptor).
- The association of leptin in the improvement of glucose metabolism during the ablation of ghrelin or the growth hormone secretagogue receptor in mice should be tested by conducting glucose kinetic measurements in mice where both ghrelin and leptin or the growth hormone secretagogue receptor and leptin are ablated. Thus, reverse genetics should be used to investigate the interplay between leptin and ghrelin or leptin and the growth hormone secretagogue receptor.
- Glucose metabolic parameters such as gluconeogenesis, glycogenolysis and insulin sensitivity as well as ghrelin concentration should be measured in morbidly obese patients before bariatric surgery, immediately after surgery and a few months after surgery. This would facilitate to explore potential mechanisms for the metabolic improvements observed immediately after bariatric surgery, i.e. before any weight loss has occurred.

