Regulation of postabsorptive glucose production in patients with type 2 diabetes mellitus
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CHAPTER 1

Introduction

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Introduction

Plasma glucose levels are controlled within relatively narrow margins. Low blood sugar levels are dangerous, because brain function is critically dependent on glucose. Conversely, if postabsorptive glucose levels are only slightly increased, diabetes mellitus is diagnosed. This diagnosis has profound implications, because it is associated with considerable morbidity and mortality. Therefore, this thesis focuses on the regulation of postabsorptive glucose production, especially in patients with type 2 diabetes mellitus.

1.1. type 2 diabetes mellitus

Diabetes mellitus encompasses different diseases with different pathogenesis, the most common variants are type 1 and type 2 diabetes. Both type 1 and 2 diabetes mellitus result in hyperglycemia and therefore share the complications of chronic hyperglycemia: retinopathy, nephropathy, and autonomic and peripheral neuropathy. In type 1 diabetes mellitus, hyperglycemia is the result of failing insulin secretion due to pancreatic beta-cell destruction, whereas in type 2 diabetes, hyperglycemia is the result of the combination of defective insulin secretion and resistance to the action of insulin, the so called insulin resistance. Of all patients with diabetes mellitus, over 90 percent has type 2 diabetes mellitus. Consequently, this disease is the most prevalent metabolic disease in the world (43). Moreover, in the last decades, the incidence and prevalence of type 2 diabetes mellitus is increasing (2). In the Netherlands, the prevalence of type 2 diabetes mellitus in elderly Caucasians recently appeared to be 8.4 % (42).

The primary causes of type 2 diabetes are unknown, but as mentioned earlier, the syndrome is characterized by insulin resistance and a relative failure of insulin secretion by the pancreatic β cell. The debate remains whether insulin resistance or a dysfunctional secretion is the primary cause of the disease (18;21). Some studies indicate that the earliest observed defect is dysfunctional secretion (31;48), whereas other publications state that a defect in insulin action is the predominant abnormality in the early stages of the development of the disease (14;53). The first detailed longitudinal study among a population with the highest documented prevalence of type 2 diabetes in the world, the Pima Indians of Arizona, confirmed the development early in the pathogenesis of type 2 diabetes of both defects in insulin action as well as in insulin secretion (72). Since insulin resistance is a consistent finding in patients with type 2 diabetes (3), and it
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Precedes the onset of type 2 diabetes by more than ten years (37), it is thus clear that insulin resistance has an crucial role in the development of type 2 diabetes mellitus.

This resistance to the action of insulin becomes apparent in those tissues that are dependent of insulin for glucose disposal (skeletal muscle and fat) or glucose production (mainly the liver). In skeletal muscle, the stimulatory effect of insulin on muscle glycogen synthesis is decreased (38). Recently, it has been shown that impaired insulin-stimulated glucose transport is the rate limiting step responsible for this decrease in skeletal muscle glycogen synthesis (7). Glucose transport into adipose tissue is quantitatively less than into muscle, but the same mechanism is thought to be responsible for the adipose tissue resistance to insulin. The major site for glucose production is the liver, and in type 2 diabetic patients there is resistance to the ability of insulin to acutely suppress hepatic glucose production (9;36). Thus, in type 2 diabetes mellitus, insulin resistance results in hyperglycemia through diminished peripheral glucose uptake and insufficient suppression of endogenous glucose production.

1.2. glucose metabolism in healthy humans

In healthy humans, plasma glucose concentration is tightly controlled: it remains at about 5 mmol/l throughout the day, rising only transiently and slightly after a carbohydrate containing meal. This fine-tuning of plasma glucose concentration occurs through adaptations in the rate of delivery of glucose to the systemic circulation (= rate of appearance of glucose, Ra) and the rate of glucose uptake by the tissues (= rate of disappearance, Rd). This is possible mainly by alterations in glucoregulatory hormones, mainly insulin and glucagon, and through the autonomic nervous system (20). Glucose uptake is either independent of insulin, like in the brain, or dependent on the action of insulin, mainly in muscle and adipose tissue. In contrast to muscle and fat, the brain cannot produce glucose, since glucose is not stored in the brain. In the postabsorptive state, therefore, brain function is critically dependent on the circulating concentrations of glucose. Since in the postabsorptive state ~ 2 mg/kg/min glucose is taken up in the body (20), the same amount of glucose has to be produced to maintain extracellular glucose concentration between its narrow ranges.

In the postabsorptive state, the production of glucose is mainly produced by the liver, but the kidney is also capable of glucose release (5;64;65). It remains
controversial, however, whether the kidney has a significant role in the production of glucose in the nonfasting nonacidotic condition. Some authors suggest that about 25% of total glucose production after an overnight fast is derived from the kidney. Other authors using the arteriovenous balance technique across the kidneys and the splanchnic area combined with intravenous infusion of $[U^{13}C_6]$-glucose, $[3-^3H]$glucose, or $[6-^3H]$glucose, estimated the renal contribution to total glucose production in the overnight fasted state to be only ~5%. During prolonged fasting, however, renal glucose production becomes substantial, comprising 20-25% of total glucose production after 60 h of fasting (17). In this thesis the term endogenous glucose production is used, which includes both hepatic and renal glucose production.

Endogenous glucose production is the resultant of two pathways: direct delivery of stored glucose (glycogen), a process called glycogenolysis, and of newly synthesized glucose molecules from different precursors, like aminoacids or lactate, called gluconeogenesis.

Under basal conditions, basal endogenous glucose production is regulated by fluctuations in portal vein insulin concentrations (61;62). An increase in portal vein insulin concentrations inhibits endogenous glucose production whereas stimulation of glucose production occurs when portal vein insulin concentration decreases. After a (carbohydrate containing) meal, both insulin and glucagon regulate glucose production. First, insulin secretion is stimulated and endogenous glucose production is inhibited. The latter is the result of inhibition of glycogenolysis by insulin as well as by the increased plasma glucose concentration. After absorption of the meal, the plasma glucose concentration decreases to values frequently below those of short-term fasting. This relative hypoglycemia is sufficient to increase the secretion and the portal concentration of glucagon, which triggers an increase in glycogenolysis and hepatic glucose production (68). Recently, it was found in healthy humans, that under hypogluconegenic conditions, the inhibitory effect of insulin on net glycogenolysis is through stimulation of glycogen synthase, whereas inhibition of glycogen phosphorylase occurs by the increased plasma glucose concentration (47). Thus, both hormonal and substrate signals are simultaneously required to promote optimal rates of net hepatic glycogen synthesis.

Endogenous glucose production however is not only dependent on gluconeogenic precursor supply or glucoregulatory hormones. Infusion of gluconeogenic precursors like glycerol, amino acids or lactate increase
gluconeogenesis, but fail to increase overall glucose production (25;28;74). These observations have led to the concept that endogenous glucose production is autoregulated, i.e. remains constant irrespective of variations in gluconeogenic flux.

Various mechanisms have been proposed to account for this constancy of endogenous glucose production. Some of them have been proven to be not true. Autoregulation is not dependent on changes in the concentrations of major glucoregulatory hormones since it persists when plasma concentrations of insulin and glucagon are maintained constant by infusions of somatostatin, insulin and glucagon (28;67). Autoregulation is also present during administration of a beta-adrenoreceptor antagonist propranolol, indicating that changes in beta-adrenergic activity are not responsible for this adaptation (22). Potential mediators of hepatic autoregulation are Kupffer cell products and the autonomous nervous system. In the liver, there is intensive interaction between Kupffer cells and hepatocytes, and in vitro animal data suggest that products of these Kupffer cells influence glucose production by hepatocytes. For instance, stimulated Kupffer cells produce prostaglandins (13), cytokines (13;39), and nitric oxide (NO) (4;39), and all these mediators can affect glucose production (4). Indomethacin influences the secretion of different mediators: prostaglandins, cytokines, as well as NO. Administration of indomethacin stimulates endogenous glucose production in healthy adults without any influence on the plasma levels of glucoregulatory hormones, insulin as well as C-peptide (12). These data suggest that intrahepatic produced mediators could influence endogenous glucose production via paracrine mechanisms.

In type 2 diabetes mellitus, there is a loss of the fine-tuning of plasma glucose concentration because of a dysfunctional secretion of insulin, as well as a resistance to the action of insulin. The adaptations in the rate of delivery of glucose to the systemic circulation and/or the rate of glucose uptake by the tissues are insufficient, and postprandial and fasting hyperglycemia is the result. Thus, in type 2 diabetes mellitus, endogenous glucose production is inappropriately increased, considering normal or even elevated insulin concentrations. The underlying mechanisms, however, still remain to be understood.

1.3. regulation of endogenous glucose production in type 2 diabetes mellitus

Despite the presence of hyperinsulinemia and hyperglycemia, basal endogenous glucose production in type 2 diabetes is increased, in the
postabsorptive state as well as in the postprandial state. There is a striking linear positive relationship between the rate of basal endogenous glucose production and the degree of fasting hyperglycemia: the higher the fasting plasma glucose concentration, the higher is the rate of endogenous glucose production (15;19;26). Although absolute rates of glucose production in patients with fasting plasma glucose concentration <10 mmol/L are not increased compared to healthy controls, comparable rates of glucose production reflect inappropriately increased rates of glucose production considering the presence of hyperinsulinemia and hyperglycemia in type 2 diabetics.

Several factors are thought to be responsible for this increase in endogenous glucose production.

1) hyperglucagonemia: significantly higher fasting plasma glucagon levels are present in patients with type 2 diabetes compared to control subjects, whereas glucose induced suppression of glucagon secretion is reduced (1;54;71). Moreover, administration of glucagon after infusion of somatostatin increases hepatic glucose production as well as plasma glucose concentration in the absence of insulin (27). A recent prospective study in non-diabetic women indeed demonstrated that high glucagon secretion (measured as response to intravenous administration of arginine) predicts glucose intolerance (35)

2) increased availability of gluconeogenic substrates: In patients with type 2 diabetes mellitus the delivery of gluconeogenic substrates to the liver is increased, as well as gluconeogenic efficiency of the liver (10;11). However, modulation of delivery of gluconeogenic substrates does not alter hepatic glucose production or plasma glucose levels. For instance, when lipolysis in patients with type 2 diabetes was inhibited by acipimox, with concomitant decrease in plasma FFA and glycerol levels, fasting hyperglycemia or the rate of endogenous glucose production does not alter, although gluconeogenesis decreases (52). In accordance, inhibition of gluconeogenesis by ethanol also reduces gluconeogenesis from endogenous precursors, but does not alter endogenous glucose production or plasma glucose concentration (60). Thus, in type 2 diabetes, the inappropriately increased endogenous production of glucose is not merely the result of increased hepatic delivery of gluconeogenic substrates, but other factors must be involved.

3) hyperglycemia: glucose itself is able to promote its own disposal and to inhibit endogenous glucose production in the presence of basal concentrations of insulin. For instance, hyperglycemia per se inhibits glucose production in nondiabetic individuals (56). Recently, an impairment in this regulation of glucose production
by glucose per se was found in patients with type 2 diabetes mellitus. In that study, somatostatin was infused in patients with type 2 diabetes and healthy, matched controls, in the presence of basal replacement of glucoregulatory hormones and plasma glucose was maintained at either 5 or 10 mmol/l. In the presence of identical and constant plasma concentrations of insulin, glucagon and growth hormone, an equivalent increase in circulating glucose concentrations (from 5 to 10 mmol/l) inhibited endogenous glucose production by 42% in healthy controls, but failed to lower endogenous glucose production in the diabetic patients (40). Thus, the autoregulatory effect of hyperglycemia is decreased in type 2 diabetes mellitus. However, because the mechanisms by which hyperglycemia per se affects endogenous glucose production in healthy subjects have not been completely elucidated, the reason why the autoregulatory effect of hyperglycemia is decreased in type 2 diabetes mellitus is also unclear at present.

4) altered regulation by paracrine mechanisms: The possible influence of intrahepatic paracrine mechanisms on endogenous glucose production was demonstrated by our group in healthy humans (12), and further confirmed in patients with uncomplicated falciparum malaria, in whom the already increased basal endogenous glucose production could be increased even more by indomethacin without any change in plasma glucoregulatory hormones or circulating cytokines (16). Thus, in healthy adults as well as in patients with certain infectious diseases, basal endogenous glucose production is not maximally stimulated, but is partially inhibited, possibly by paracrine factors like prostaglandin’s, cytokines and/or nitric oxide. Consequently, it is possible, that these paracrine factors also influence endogenous glucose production in other conditions like in type 2 diabetes mellitus. If this is the case, dysregulation of paracrine interaction could be important co-factor in maintaining increased endogenous glucose production in type 2 diabetes mellitus.

5) the influence of diets and meal composition: nutritional intake itself is an important determinant of the rate of postabsorptive endogenous glucose production. In healthy humans, there is a direct relation between carbohydrate intake and postabsorptive endogenous glucose production (58). Carbohydrate overfeeding increases postabsorptive glucose production (8), whereas fasting reduces glucose production (30). A deterioration in carbohydrate metabolism could be induced by a “modern” high fat diet in non diabetic Pima Indians, a population with high prevalence of type 2 diabetes, as well as in non diabetic Caucasians (66). No data are available on the potential role of eucaloric changes in dietary content in
the regulation of glucose production in healthy subjects. As FFA stimulates gluconeogenesis (6), it is quite possible that fat will stimulate glucose production (and via this way contribute to the development of type 2 diabetes mellitus), even in the absence of the induction of adiposity.

**1.4. methods for quantifying endogenous glucose production and gluconeogenesis**

1.4.1. **estimation of endogenous glucose production**

Isotopes of glucose are used for the estimation of endogenous glucose production in humans *in vivo*. The procedures involve primed, continuous infusion of labeled glucose. Both radioactively labeled glucose, e.g. [3-³H]glucose, as well as stable isotopes of glucose, e.g. [6,6-²H₂]-glucose can be used for quantification of endogenous glucose production (73). These isotope dilution methods require assumptions regarding the distribution volume of glucose, the presence of steady state of the isotope at the time of calculation, and of characteristics of the behavior of the glucose molecules in one or more pools.

When isotopic steady state is reached, i.e. when glucose specific activity (radioactive isotope) or the tracer/tracee ratio of glucose (stable isotope) does not change during a certain time, the endogenous production of glucose can be calculated using steady state equations according to Steele (63):

\[
R_a = \frac{F}{E}
\]

where \( R_a \) = the rate of appearance of glucose (in \( \mu \text{mol/kg/min} \)), \( F \) = tracer infusion rate (in \( \mu \text{mol/kg/min} \)) and \( E \) = percent of glucose molecules enriched with \(^2\text{H}\).

The purpose of the priming dose is to instantaneously label the whole glucose pool to the tracer steady state level that would eventually be reached with the constant infusion alone. In healthy subjects, reliable calculations of \( R_a \) can be made using the steady state equations, two hours after a fixed priming dose of the tracer. Isotopic tracer equilibrium has than been achieved.

Major differences in absolute basal rates of endogenous glucose production were reported in patients with type 2 diabetes mellitus, varying from normal rates (e.g. similar as in healthy subjects) to 140% higher than normal (15;57). In 1990, a
study was conducted in type 2 diabetic patients in the overnight fasted state to elucidate whether these differences could be due to the mode of priming, fixed or adjusted to the prevalent hyperglycemia, and/or to the mode of calculation: steady state or non-steady state equations (24). Between 10-16 h of fasting, plasma glucose concentration was not constant, but declined ~0.5 mmol/l/h. Furthermore, using fixed priming, tracer steady state was not reached within 6 h, whereas using adjusted priming a constant tracer steady state was obtained within 60 min. Thus, the fasting state in patients with type 2 diabetes mellitus is not a steady state condition and consequently, using Steele's equations after fixed priming, glucose production rates calculated after 2 h will be overestimated in proportion to fasting hyperglycemia. Thus, in patients with type 2 diabetes mellitus a prime, adjusted to the prevalent hyperglycemia has to be administered and calculations have to be performed assuming non-steady state.

Modifications of the formula of Steele (63) allow us to calculate the rate of appearance of glucose under non-steady state conditions:

$$ R_a = \frac{F - pV \frac{(C_1 + C_2)}{2} \frac{(E_2 - E_1)}{(t_2 - t_1)}}{(E_2 + E_1) \frac{2}{2}} $$

where $R_a$ = rate of appearance of glucose (in μmol/kg/min),

$F$ = tracer infusion rate (in μmol/kg/min)

$E$ = percent of glucose molecules enriched with $^2$H (in absolute values)

$C$ = plasma glucose concentration (in mmol/L)

$t$ = time point for measurement

$pV$ = effective distribution volume of glucose

This non-steady state equation of Steele is based on several assumptions:

1) the presence of a single, well mixed glucose pool in our body.
2) uniform and instantaneous mixing of the infused glucose tracer with the unlabeled glucose pool.
3) once glucose has left the glucose pool, no glucose molecule will reenter the pool.
It was recognized by Steele that glucose being sampled in the (plasma) pool, does not mix instantaneously with the total body glucose pool. He therefore suggested to multiply the distribution volume of glucose (V) by a pool correction factor p, a fudge factor to define an effective volume of distribution of glucose (pV) and to compensate for the use of calculations based on a single-pool model in a system that is actually multicompartimental. For this reason, and because pV may change in time during non-steady state, the rate of appearance can be calculated by using different values of pV, ranging from the smallest plausible volume (e.g. the plasma volume) to the largest one (e.g. interstitial volume) in order to approximate bounds of the true value.

1.4.2. *quantification of gluconeogenesis and glycogenolysis*

Endogenous glucose production consists of two components: gluconeogenesis and glycogenolysis. Since the introduction of isotopes for estimation of molecular fluxes, several methods have been developed to measure the contribution of gluconeogenesis and glycogenolysis to endogenous glucose production:

In the seventies, several methods were introduced involving measurement of arteriovenous differences across the splanchnic area. This technique involves splanchnic catheterization and measurements of arterial as well as venous concentrations of gluconeogenic substrates. By multiplying the difference between arterial and venous concentrations by the hepatic blood flow (measured by indocyanine green) (69), gluconeogenesis can be calculated. However, calculating splanchnic net balance does not account for hepatic uptake of substrates formed within the splanchnic bed, like the intestinal release of amino acids and lactate, nor do they allow for splanchnic extra-hepatic glucose utilization and the renal contribution to endogenous glucose production (70). Moreover, the large variation coefficient of the flow measurements precluded the detection of small arterio-venous differences in substrate concentrations.

A simple and non-invasive method for quantifying gluconeogenesis is the infusion of different radioactive and stable isotopes of precursors of gluconeogenesis. However, the application of labeled precursors of gluconeogenesis like lactate, alanine and pyruvate suffer from the limitation that these tracers are diluted in the rapidly turning over oxaloacetate pool, before its conversion to glucose. This oxaloacetate pool cannot be measured directly, but has to be taken into account when measuring the enrichment of the precursor pool for
gluconeogenesis, before gluconeogenesis can be calculated (41). Moreover, isotopic exchanges in the oxaloacetate pool result in uncertain dilution of the labels (59). As a result, all these stable isotope approaches are limited by uncertain assumptions regarding the enrichment of this oxaloacetate pool.

A totally different method for the estimation of gluconeogenesis was introduced in the early nineties by Shulman et al. (55). In contrast to the abovementioned techniques, this method directly measures glycogenolysis, by quantification of changes in hepatic glycogen, applying NMR spectroscopy in combination with magnetic resonance imaging (MRI) of liver volume in order to calculate the depletion of hepatic glycogen. Gluconeogenesis can then be calculated by subtracting the rate of net hepatic glycogenolysis from the rate of endogenous glucose production as measured by $^3$H-glucose. Gluconeogenesis thus is not measured directly but depends on an estimate of the difference in hepatic glycogen content. Moreover, as mentioned earlier, endogenous glucose production comprises hepatic, as well as renal glucose production, whereas glycogenolysis with this method is only measured from the liver.

Three different stable isotope methods for quantification of gluconeogenesis in vivo have been described, that do not involve the assumptions regarding the enrichments within the oxaloacetate precursor pool. The first method was introduced by Hellerstein and co-workers, who applied mass isotopomer distribution analysis (MIDA) as a method for estimating the fractional synthetic rate of various biopolymers, including cholesterol, fatty acids, glucose, and DNA (23). Glucose is considered as a dimer formed from the condensation of two triose phosphate molecules. Thus, MIDA of glucose made from a $^{13}$C-labeled gluconeogenic precursor (infused as [2-$^{13}$C]glycerol or [U$^{13}$C$_3$]glycerol) has been proposed as a method for estimating the contribution of gluconeogenesis ($f$) to total endogenous glucose production (44). These MIDA calculations of $f$ are not subject to artifacts of isotope exchange or dilutions, provided the main underlying assumption of MIDA is fulfilled, that is: the triose phosphate pool(s) in all gluconeogenic cells must be at similar $^{13}$C enrichments, otherwise $f$ will be underestimated (32;50;51).

There are, however, conflicting opinions regarding the general applicability of MIDA for estimating $f$ during the infusion of $[^{13}$C]glycerol in vivo. Several investigators have infused [2-$^{13}$C]glycerol and concluded that correct estimates of $f$ was possible (44-46), whereas others infused [U$^{13}$C$_3$]glycerol and concluded that $f$ was underestimated and that MIDA is not a reliable method for estimating $f$
(32;50). A recent publication has shown that in *vitro* the relative contribution of \(^{13}\text{C}\)glycerol versus other gluconeogenic precursors influences the determination of \(f\), such that \(f\) increases as the contribution of \(^{13}\text{C}\)glycerol increases. Moreover, glucose production increases as the supply of \(^{13}\text{C}\)glycerol increases (51). These substrate induced effects of \(^{13}\text{C}\)glycerol infusion on glycerol and glucose metabolism were further confirmed in *in-vivo* experiments in 30 h fasted mice. Estimates of \(f\) by MIDA yielded erroneous results with low infusion rates of \([2-{^{13}\text{C}}]\)glycerol, whereas reasonable estimates of \(f\) were obtained at glycerol infusion rates that perturb glycerol and glucose metabolism (49).

A modification of MIDA to quantify gluconeogenesis, based on the use of \([\text{U-}^{13}\text{C}]\text{glucose}\) was published by Tayek and Katz (29), but proved underestimate gluconeogenesis, because underlying assumptions could not be fulfilled, and because the contribution of gluconeogenesis from glycerol and amino acids not metabolized was ascribed to glycogenolysis (34).

The third method was introduced by Landau et co-workers, using the oral administration of \(^2\text{H}_2\text{O}\) with subsequent measurement of the enrichment of deuterium in specific positions of glucose (32;33). Because the exchange of deuterium between the gluconeogenic precursors and body water occurs after passing through the oxaloacetate pool, this method also does not involve the limitations of the unknown enrichment of this pool. The approach rests on the fact that hydrogen bound to carbon 5 of glucose formed by gluconeogenesis, in the conversion of phosphoenolpyruvate to 2-phosphoglyceraldehyde, has water as its source. Furthermore, when glycerol is converted to glucose, carbon 5 of the glucose is from carbon 2 of glycerol. Hydrogen from water is transferred to that carbon in the isomerization of dihydroxyacetone-3-P from the glycerol with glycerol-3-phosphate-3-P, and that isomerization is extensive. In glycogenolysis on the contrary, there is no exchange with water of the hydrogen bound to carbon 5 of the glucose formed. Thus, the ratio of enrichment at carbon 5 of glucose to that at carbon 2, or in water at steady state, is a direct measure of the fraction of glucose formed by gluconeogenesis. A number of possible hydrogen exchange reactions, however, can also occur that would not represent true gluconeogenesis, like the exchange of \(^2\text{H}\) into fructose-1,6-diphosphate (FDP) in the process of incomplete FDP aldolase cleavage reaction. This would result in overestimation of the fractional contribution of gluconeogenesis.
Thus, although new methods have been developed that get round the problems of the oxaloacetate precursor pool enrichment, so far no method can be considered to be the gold standard for measurements of gluconeogenesis.

1.5. outline of the present thesis

Postabsorptive endogenous glucose production in patients with type 2 diabetes mellitus is inappropriately increased as result of resistance to the suppressive action of insulin on the liver. The cause of this hepatic insulin resistance in type 2 diabetes mellitus is unknown. Other factors like hyperglucagonemia, increased availability of gluconeogenic substrates or autoregulatory effect of glucose can not adequately explain this increase in postabsorptive glucose production. Recent studies indicate that in healthy subjects intrahepatic paracrine factors can influence basal endogenous glucose production. It is currently unknown, if these paracrine regulators also influence basal endogenous glucose production in type 2 diabetes mellitus.

The objective of this thesis was to obtain more insight in the regulation of endogenous glucose production in the postabsorptive state in patients with type 2 diabetes mellitus, with a focus on the possible role of paracrine factors, and diet and in the relative contribution of gluconeogenesis and glycogenolysis to total glucose production in the postabsorptive state.

Research questions:
A] Role of paracrine factors in the induction of changes in endogenous glucose production

Prostaglandin’s are products of stimulated Kupffer cells that can stimulate glucose production in hepatocytes. Indomethacin influences the secretion of these mediators and administration of indomethacin to healthy volunteers stimulates basal endogenous glucose production without any changes in glucoregulatory hormone concentrations. If the same holds true for patients with type 2 diabetes mellitus is one of the research questions. It is also known that indomethacin can potentially inhibit glucose stimulated insulin secretion. The second research question was therefore: is an effect of indomethacin on glucose production dependent on the ambient plasma insulin concentration?
Adenosine is another paracrine regulator which can be formed and released in tissues, including the liver. Administration of pentoxifylline, an adenosine receptor antagonist, inhibited transiently endogenous glucose production in healthy humans without any changes in glucoregulatory hormone concentrations. To evaluate the possible modulatory role of adenosine on endogenous glucose production in type 2 diabetes, aminophylline, a potent adenosine receptor antagonist, was administered intravenously to type 2 diabetic patients.

B] Role of nutritional substrate in the induction of changes in endogenous glucose production and gluconeogenesis

Nutritional intake is an important determinant of the rate of postabsorptive glucose production (58). Changes in post-absorptive glucose production reflect changes in gluconeogenesis and/or glycogenolysis, because endogenous glucose can only be derived from gluconeogenesis and glycogenolysis. Quantification of these two pathways is essential for better understanding of changes in intra-hepatic glucose metabolism induced by variations in carbohydrate intake. We therefore quantified gluconeogenesis (by ingestion of $^2$H$_2$O) and glycogenolysis after 11 days of a high carbohydrate (85% carbohydrate), control (44% carbohydrate) and very low carbohydrate (2% carbohydrate) diet in six healthy males. Diets were eucaloric and provided 15% of energy as protein. Post-absorptive endogenous glucose production was measured by infusion of [6,6-$^2$H$_2$]glucose.

C] Measurement of gluconeogenesis in vivo in humans

The quantification of gluconeogenesis by two new methods: the administration of $^2$H$_2$O and by [2-$^{13}$C]glycerol and the mass isotopomer distribution analysis (MIDA) of glucose, does not involve assumptions regarding the enrichment of the oxaloacetate pool. Both methods are used as a golden standard for measurement of gluconeogenesis in vivo, but it is currently unknown if both methods give identical results. The relative value of each method was tested by comparing these two methods in healthy postabsorptive volunteers under identical, strictly standardized eucaloric conditions on three separate occasions: once after oral administration of $^2$H$_2$O, once during a primed, continuous infusion of [2-$^{13}$C]glycerol, and once during a primed continuous infusion of unlabeled glycerol after oral administration of $^2$H$_2$O to investigate the possible influence of glycerol infusion on glucose production and gluconeogenesis measurements.
Changes in endogenous glucose production and gluconeogenesis during short-term fasting.

In healthy subjects, endogenous glucose production adapts to short term starvation (< 24 h) by a decrease in glycogenolysis, whereas gluconeogenesis does not change. In type 2 diabetes mellitus plasma glucose concentration decreases faster during short term starvation. It is unknown if this difference in changes in plasma glucose over time between healthy subjects and patients with type 2 diabetes mellitus is reflected in comparable changes in glucose production and gluconeogenesis. To evaluate the adaptation of glycogenolysis and gluconeogenesis to a short extension of the postabsorptive state, we compared in patients with type 2 diabetes mellitus plasma glucose concentration, endogenous glucose production and gluconeogenesis between 16 to 20 hours of fasting versus between 20 to 24 hours of fasting. Endogenous glucose production was measured by infusion of [6,6-\textsuperscript{2}H\textsubscript{2}]glucose, and gluconeogenesis by administration of \textsuperscript{2}H\textsubscript{2}O.
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