Regulation of postabsorptive glucose production in patients with type 2 diabetes mellitus
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CHAPTER 8

General Discussion and Conclusions
Type 2 diabetes mellitus is characterized by postabsorptive hyperglycemia and increased endogenous glucose production. There is a positive correlation between the rate of endogenous glucose production and the degree of postabsorptive hyperglycemia: the higher fasting plasma glucose concentration, the higher the rate of endogenous glucose production (8;10;13). Factors like hyperglucagonemia, increased availability of gluconeogenic substrates or autoregulatory effects of glucose can not adequately explain this increase in postabsorptive glucose production. Previous studies in healthy subjects indicated that intrahepatic paracrine factors may 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 studies in this thesis show that the regulatory mechanisms of postabsorptive endogenous glucose production in type 2 diabetes mellitus differ from those in healthy humans.

8.1. The role of paracrine factors

Administration of indomethacin, a prostaglandin synthesis inhibitor, to patients with type 2 diabetes increases endogenous glucose production to a similar extend as previously reported by our group in healthy volunteers (by ~50% from basal) (6). In contrast, however, to the data in healthy volunteers, in type 2 diabetic patients insulin secretion was inhibited at the same time by ~50%. When indomethacin was administered to these patients together with somatostatin in combination with insulin and glucagon in order to clamp insulin and glucagon concentrations at basal levels, the stimulatory effect of indomethacin on endogenous glucose production was no longer found. This proves that the stimulatory effects of indomethacin on hepatic glucose production in type 2 diabetes mellitus are caused by inhibition of insulin secretion. Superficially, this may seem the most obvious conclusion. An alternative possibility has to be considered: namely when the modulatory role of paracrine factors is limited to situations where endogenous glucose production is only mildly deranged. In our first study without somatostatin mean endogenous glucose production increased from 11.2 to a max of 17.8 μmol.kg.min⁻¹, whereas basal endogenous glucose production in our study with somatostatin was already 16 μmol.kg.min⁻¹ prior to administration of indomethacin. It is, therefore, still possible that the influence of paracrine regulation of glucose production is only apparent when glucose production is between 10 and 16 μmol.kg.min⁻¹. A comparable possibility with a glucose regulatory system only active in a certain area of the whole system has also
been suggested for the regulation of the glucose production by the plasma glucose concentration itself (EPM Corssmit, Amsterdam Thesis Publishers 1993). Unfortunately, the studies presented in the present thesis do not permit a conclusion in this respect and additional studies are required to solve this issue.

In this thesis the original aims of the studies were focussed on the evaluation of paracrine, intrahepatic regulation of glucose metabolism by mediators like prostaglandins or cytokines. Rather, in type 2 diabetes, our data suggest that prostaglandins are involved in the regulation of the secretion of the main glucoregulatory hormone: insulin. It has been known for decades that under physiological conditions the beta-cell tonically synthesizes prostaglandin E2, a process known to be stimulated by glucose (19). On the other hand, prostaglandin E2 inhibits glucose-induced insulin secretion (17). Thus, it appears unlikely, that, although indomethacin inhibits prostaglandin synthesis, the effect of indomethacin on beta cells is due to inhibition of prostaglandin synthesis, because that would result in stimulation rather than inhibition of insulin secretion. However, in addition to inhibition of prostaglandin synthesis indomethacin also stimulates cytokine production. In healthy humans indomethacin is a potent stimulator of interleukin (IL)-1-beta, both in vitro as well as in vivo (9). IL-1 beta stimulates the generation of the inducible form of cyclooxygenase (COX-2), the enzyme responsible for generation of prostaglandin E2 from arachidonic acid(16). The effect of IL-1 can be either directly by increasing gene expression of COX-2 mRNA, or indirectly through production of nitric oxide (NO)(15). Thus, stimulation of IL-1 by indomethacin could result in inhibition of insulin secretion, through stimulation of COX-2.

Finally, inhibition of insulin secretion can be accomplished by affecting the signal-transduction pathways of the insulin receptor itself. For instance, mice with tissue-specific knockout of the insulin receptor in beta-cells, but not elsewhere in the body, develop insulin secretory defects similar to those in type 2 diabetes (14). A functional insulin receptor on beta-cells thus is a prerequisite for a normal glucose-stimulated insulin secretion. Apparently, insulin stimulates its own release by a positive feedback loop through binding to its own receptor in the beta cell. Interestingly, indomethacin can inhibit autophosphorylation of the beta subunit of the insulin receptor (5). Therefore, impairment of the function of the insulin receptor by indomethacin by inhibiting autophosphorylation of the beta subunit could lead to inhibition of insulin secretion.
Adenosine is another potential paracrine modulator of endogenous glucose production. Therefore, we evaluated the effect of modulation of adenosine activity on endogenous glucose production in type 2 diabetes mellitus. However, the same differences with respect to insulin secretion between patients with type 2 diabetes mellitus and healthy humans was found like with indomethacin (see above), when aminophylline, an adenosine receptor antagonist, was administered to type 2 diabetic patients. Administration of aminophylline resulted in a transient inhibition of endogenous glucose production similar to the effect in healthy humans associated with an increase in insulin secretion, whereas aminophylline did not affect insulin concentrations in healthy volunteers. Despite the fact that plasma insulin concentrations were stimulated in patients with type 2 diabetes, the absolute decrease in endogenous glucose production was less than in healthy volunteers, indicating that intrahepatic paracrine factors do not play a major role under conditions were insulin secretion is stimulated like in type 2 diabetes mellitus. It also indicates that basal insulin secretion is actively inhibited in patients with type 2 diabetes by mechanisms that involve factors like adenosine. This is in accordance with the only available (in vitro) study on the role of adenosine on pancreatic beta-cells: In mouse islets adenosine caused an inhibition of glucose-induced insulin release. This inhibition was no longer observed when insulin release was potentiated by cAMP (2). Since aminophylline also inhibits phosphodiesterase, it is also possible that the stimulatory effect on insulin secretion in type 2 diabetes is the result of inhibition of phosphodiesterase, resulting in increased cAMP concentrations.

In conclusion, we found no evidence for a modulatory role of intrahepatic paracrine factors in increased postabsorptive glucose production in type 2 diabetes mellitus. However, we did find evidence for altered, probably paracrine, regulation of insulin secretion in type 2 diabetes mellitus, documented by the effects of indomethacin and aminophylline on insulin secretion.

8.2. The role of changes in glycogenolysis and gluconeogenesis in postabsorptive endogenous glucose production

The quantification of gluconeogenesis by $^2$H$_2$O and by [2-¹³C]glycerol does not involve assumptions regarding the enrichment of the oxaloacetate precursor pool. Although both methods are considered as golden standard, a direct comparison of these two different approaches had not yet been performed.
When compared in healthy postabsorptive males under identical, strictly standardized, eucaloric conditions on three separate occasions, the contribution of gluconeogenesis to glucose production measured after $^2$H$_2$O administration and during [2-$^{13}$C]glycerol infusion appeared to be significantly different, representing $\sim$60 vs $\sim$41% of endogenous glucose production, respectively. Since this discrepancy is not merely caused by infusion of glycerol per se, it probably relates to conceptual problems in underlying assumptions in one or both methods. This has profound implications for the comparison of fractional and absolute gluconeogenic rates in different studies. Only data obtained with the same method of measurement should be taken in consideration.

In type 2 diabetes mellitus, the non-linear decrease of plasma glucose concentration in the postabsorptive state was preceded by a non-linear decrease in endogenous glucose production. This was initially at least in part caused by a fall in the rate of glycogenolysis, whereas later into the fast glycogenolysis only decreased minimally but a further decrease in endogenous glucose production was prevented by an increase in gluconeogenesis. This pattern of changes in glycogenolysis and gluconeogenesis differs from those previously published in healthy subjects (3;4), in whom the decrease in plasma glucose concentration and endogenous glucose production was linear, due to decreases in glycogenolysis, whereas the absolute rate of gluconeogenesis did not change.

The adaptation to short-term starvation thus differs between patients with type 2 diabetes and healthy subjects:

1) in healthy subjects a major decrease in plasma glucose is prevented by a decrease in peripheral uptake, since endogenous glucose production decreases by about 20% between 16 and 22 hours of fasting, whereas plasma glucose concentration hardly changes.

2) in patients with type 2 diabetes mellitus a decrease in plasma glucose concentration is not prevented by a decrease in peripheral glucose uptake (reflected by the metabolic clearance rate, which did no change), but by a stabilization of endogenous glucose production, through an increase in the rate of gluconeogenesis.

Changes in endogenous glucose production rather than changes in glucose uptake seem to be the major mechanism in the adaptation to short-term starvation in type 2 diabetes. Type 2 diabetes mellitus is caused by a combination of disturbances in insulin secretion and insulin resistance with respect to glucose
uptake. In healthy subjects the adaptation to short term starvation induces an
decrease in insulin sensitivity with respect to glucose uptake. This mechanism
enables to maintain plasma glucose levels within certain limits despite a decrease
in endogenous glucose production (18). It is tempting to speculate that this
mechanism of decreased insulin sensitivity is already present in type 2 diabetes
mellitus in the postabsorptive state, precluding a further adaptation during short
term starvation. Consequently, during short term starvation plasma glucose levels
in type 2 diabetes are dependent on the rate of endogenous glucose production,
rather than on the combination of decreasing glucose production and decreased
glucose uptake, like in healthy subjects.

8.3 The role of changes in postabsorptive plasma glucose concentration and the
duration of the fast in type 2 diabetes

In contrast to type 1 diabetes, the onset of type 2 diabetes is insidious and
is usually recognized only 5-12 years after hyperglycaemia develops. During this
period of undiagnosed diabetes, hyperglycaemia, in combination with lifestyle
factors, dyslipidaemia, obesity, insulin resistance and hypertension frequently
associated with type 2 diabetes, promote the initiation and progression of micro-
and macrovascular complications. This delay in diagnosing the disease results in a
high prevalence of chronic complications at the time of actual diagnosis:
cardiocvascular disease and neuropathy are found in approximately 10% of cases,
and retinopathy and nephropathy in 15-20%. It is therefore very important to
secure earlier detection that leads to fast and aggressive treatment of the
accelerated chronic complications. Since 1985, the diagnose of type 2 diabetes
mellitus was based according to the WHO criteria (12): fasting plasma glucose
concentration of >7.8 mmol/l and an impaired oral glucose tolerance test (OGTT)
(as measured 2 hours after an oral 75 gram glucose load). Recently, the American
Diabetes Association (ADA) introduced new diagnostic criteria. These new criteria
are based only on fasting plasma glucose levels, avoiding the burdensome OGTT.
Since then, in several studies the 1997 ADA criteria were compared with the 1985
World Health Organization (WHO) criteria with respect to the prevalence of
diabetes. In the Dutch population of the Hoorn Study the prevalence of diabetes
was similar for both sets of criteria, but ~40% of subjects who were diagnosed with
diabetes according to the 1997 ADA criteria were not classified as having diabetes
when using the 1985 WHO criteria. Similarly, of 285 subjects diagnosed with
impaired fasting glucose by the 1997 ADA criteria, 195 (68.4%) were classified as
having normal glucose tolerance by the 1985 WHO criteria and the overall agreement was poor (kappa 0.33; 95% CI 0.28-0.38)(7).

The fasting plasma glucose concentration is thus used as the key feature in diagnosing type 2 diabetes mellitus according to the ADA criteria. However, in a recent study comparing the 1997 ADA criteria with the 1985 World Health Organization (WHO) criteria with respect to the prevalence of diabetes in the U.S. population, the overnight fast was defined by an interval between 9 and 24 hours after the last meal! (11).

Our studies however clearly indicate that the fasting plasma glucose concentration is dependent not only on the duration of the fast (thus the time of the last meal), but also on the composition of the meal.

Figure 1 clearly illustrates that that the time of the last meal the evening before (the duration of the fast), as well as the time of sampling during the day, influences postabsorptive glucose concentration: When the last (carbohydrate) containing meal was taken at 6 pm, mean plasma glucose concentration decreased from 8 to 11 am the following morning by 1.4 mmol (from 9.3 ± 0.7 to 7.9 ± 0.5 mmol/l).

**Figure 1:** The effect of: 1) time of consumption of last evening meal and of 2) time of sampling during the day from 8 a.m. to 5 p.m. on mean (± SEM) fasting plasma glucose concentration in patients with type 2 diabetes mellitus. Closed circles: last meal 10 p.m. the evening before (n=6). Open circles: last meal at 6 pm the evening before (n=6).
In another 6 patients with type 2 diabetes plasma glucose concentration was determined after consumption of the last meal at 10 pm. Between 8 and 11 am, mean plasma glucose concentration only decreased by 0.6 mmol/l (from 10.5 ± 0.6 to 9.9 ± 0.8 mmol/l).

Thus, it is well possible, that in some patients, the diagnosis of diabetes will be missed, because the duration of the fast (time of last meal and time of sampling during the day) is not strictly defined, resulting in a prolonged fasting period. Since the diagnosis has such profound implications with respect to mortality and morbidity, we recommend standardization of the fasting period involved in the measurement of fasting plasma glucose concentrations in individuals screened for impaired glucose tolerance or diabetes mellitus.

8.4. Implications for the treatment and future research in patients with type 2 diabetes mellitus

The UKPDS study clearly indicates that the standard therapy for patients with type 2 diabetes mellitus is not extremely effective, even when it is applied as strict as possible, as the HbA₁C levels deteriorates over the years(1). Apparently, type 2 diabetes mellitus is a progressive disease, irrespective of therapy. Therefore, alternative approaches are warranted. As increased glucose production is one of the hallmarks of type 2 diabetes, the possibility of alternative explanations for this disturbance with concomitant new possibilities for drug treatment are worthwhile to explore. Drugs that modulate the production of prostaglandin’s, adenosine and possible other mediators will not be very effective drugs for the treatment of hyperglycemia, not only because dysregulation at the level of Kupffer cell-hepatocyte interaction is at most only a minor mechanism in the induction of hyperglycemia in type 2 diabetes mellitus, but also because these mediators are involved in the regulation of the glucose-stimulated insulin secretion, an other hallmark of this type of diabetes.

The difference in the response of endogenous glucose production between healthy subjects and type 2 diabetics in the adaptation to short-term starvation suggest that it is worthwhile to explore the pathophysiological background of this difference, particularly because basal endogenous glucose production is the main determinant of fasting plasma glucose concentration. The role of diet in this aspect is an important one to explore in this disease as the data in chapter 6 clearly
indicate that even in healthy subjects eucaloric changes in the composition of a diet have major influences on endogenous glucose production and its components.
Reference List


152


