Hepatic Insulin Clearance in Regulation of Systemic Insulin Concentrations—Role of Carbohydrate and Energy Availability

Kirstine N. Bojsen-Møller,[1,2] Anne-Marie Lundsgaard,[3] Sten Madsbad,[1,2] Bente Kiens,[3] and Jens Juul Holst[2,4]

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Hyperinsulinemia is the hallmark of insulin resistance in obesity, and the relative importance of insulin clearance, insulin resistance, and insulin hypersecretion has been widely debated. On the basis of recent experimental evidence, we summarize existing evidence to suggest hepatic insulin clearance as a major and immediate regulator of systemic insulin concentrations responding within days to altered dietary energy and, in particular, carbohydrate intake. Hepatic insulin clearance seems to be closely associated with opposite alterations in hepatic lipid content and glucose production, providing a potential mechanistic link to hepatic insulin sensitivity. The molecular regulation of insulin clearance in the liver is likely to involve changes in insulin binding and receptor internalization in response to the dietary alterations, the molecular mechanisms of which await further research.

Hyperinsulinemia is the hallmark of insulin resistance in obesity. Still, the pathogenesis of hyperinsulinemia has been debated for decades, in particular with respect to the interplay between insulin hypersecretion and insulin resistance. It is generally assumed that upregulation of insulin secretion compensates for insulin resistance (1), but it has also been suggested that insulin resistance develops secondary to insulin hypersecretion (2). The relative importance of peripheral versus hepatic insulin resistance and the role of insulin clearance have, however, received less attention (3). Insulin is cleared mainly by the liver, and hepatic insulin clearance therefore contributes to regulate insulin action by controlling insulin availability to peripheral tissues. Recently, reduced hepatic insulin clearance has been suggested as the initial driver for systemic hyperinsulinemia in obesity, while insulin hypersecretion was observed in more advanced stages of insulin resistance (4,5). In accordance, we reported reduced insulin clearance in healthy lean subjects after 3 days on a diet with high carbohydrate (80E%) and low fat (9E%) content and a 75% increase in daily energy provision (6), whereas energy restriction induced by Roux-en-Y gastric bypass (RYGB) markedly increased insulin clearance within 1 week in obese subjects with normal glucose tolerance and obese patients with preoperative type 2 diabetes (T2D) (7) (Fig. 1*A* and *B*). Interestingly, in the dietary study, 3 days with high dietary fat (78E%) and reduced carbohydrate (10E%) intake under conditions of matched 75% caloric excess led to increased insulin clearance (6) (Fig. 1*A*), pointing toward carbohydrate availability rather than energy availability as the major regulator of insulin clearance. In both studies, the up- or downregulation of insulin clearance occurred in parallel with opposite changes in basal hepatic glucose production, as assessed by glucose tracer infusion (Fig. 1*C* and *D*), suggesting a mutual interaction between insulin clearance and insulin sensitivity of the liver. Notably, the changes in insulin clearance and hepatic glucoregulation occurred independently of changes in peripheral insulin sensitivity as measured by the hyperinsulinemic-euglycemic clamp (6).

These observations suggest that insulin clearance in the liver is rapidly modified under conditions of changed

[1]Department of Endocrinology, Copenhagen University Hospital Hvidovre, Hvidovre, Denmark
[2]Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark
[3]Section of Molecular Physiology, Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Copenhagen, Denmark
[4]Department of Biomedical Sciences, Faculty of Health, University of Copenhagen, Copenhagen, Denmark

Corresponding author: Kirstine N. Bojsen-Møller, kirstine.bojsen-moeller@regionh.dk.

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K.N.B.-M. and A.-M.L. share first authorship.

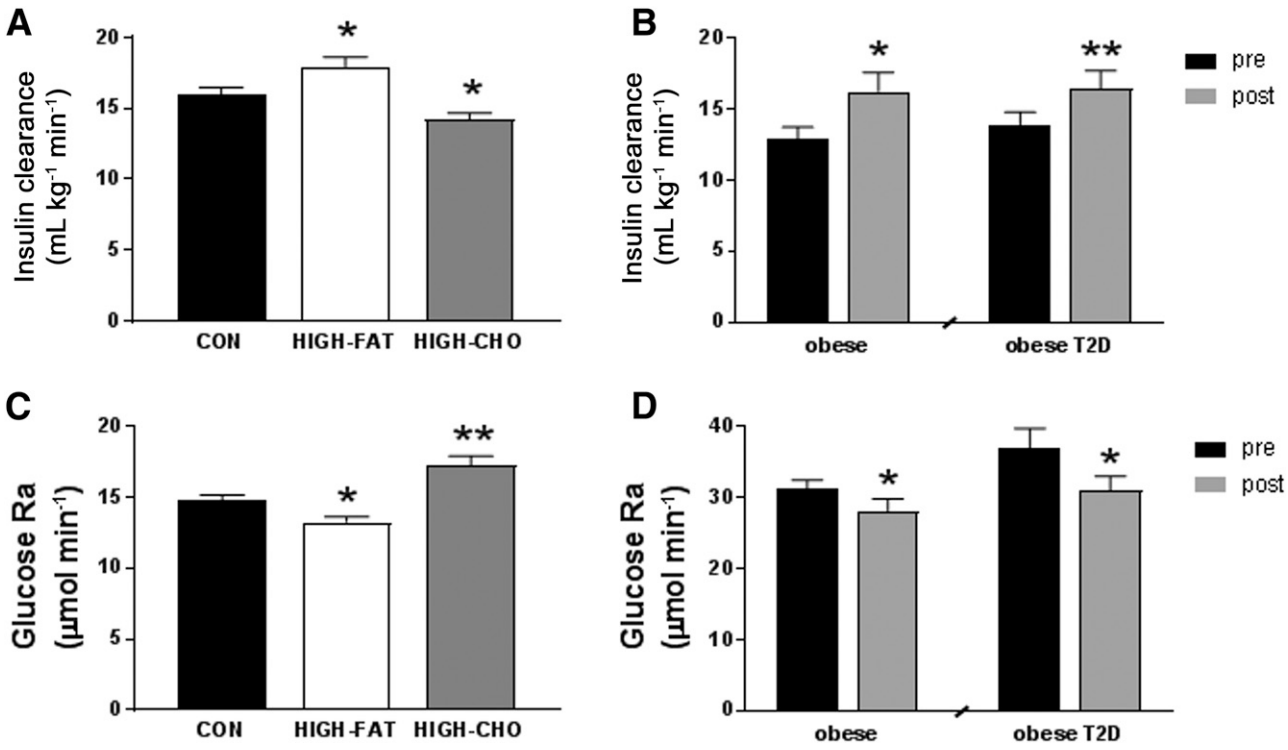

Figure 1—Insulin clearance and basal hepatic glucose production after 3 days manipulation with macronutrient and energy intake in healthy young subjects (*A* and *C*) and before (pre) and 1 week after (post) RYGB in obese subjects with preoperative normal glucose tolerance (obese) or T2D (obese T2D) (*B* and *D*). Data were previously published in Lundsgaard et al. (6) and Bojsen-Møller et al. (7). Insulin clearance was calculated from hyperinsulinemic-euglycemic clamps with insulin infusion rates of 56 and 40 mU/m^2/min, respectively. Glucose rate of appearance (Ra) was calculated from the results of a 2-h primed continuous basal infusion of 6,6-2H2 glucose tracer (0.055 and 0.036 mg/kg/min, respectively). CON: eucaloric diet (13.7 ± 0.2 MJ/day) with 65E% carbohydrate, 14E% fat, and 24E% protein. HIGH-FAT: 3-day hypercaloric diet (24.0 ± 0.4 MJ/day) with 10E% carbohydrate, 78E% fat, and 12E% protein. HIGH-CHO: 3-day hypercaloric diet (24.0 ± 0.4 MJ/day) with 80E% carbohydrate, 9E% fat, and 11E% protein. Data are means ± SE. For *A* and *C*, $n = 9$ except for $n = 8$ in HIGH-CHO. For *B* and *D*, $n = 8$ in each group. *$P < 0.05$, **$P < 0.01$ compared with CON or pre.

dietary carbohydrate intake. In the following, we summarize the evidence supporting reduced hepatic insulin clearance induced by acute increase in energy and carbohydrate availability as an important mediator in the development of systemic hyperinsulinemia.

INSULIN CLEARANCE—SITE AND MEASUREMENT

The liver is the major site of insulin clearance. Splanchnic extraction of peripherally administered insulin is 50–70% when directly measured by the hepatic venous catheter technique, and this represents almost exclusively hepatic clearance (8–10). For endogenous insulin, hepatic extraction is of even greater importance due to the direct delivery to the portal vein (11,12). The kidneys are the main site of extrasplanchnic insulin clearance, with additional contributions resulting from uptake and degradation by peripheral insulin-sensitive tissues, i.e., skeletal muscle and adipose tissue (10). Renal clearance is, however, modest (~15–25%) under physiological conditions (10), and even in patients with end-stage renal failure, systemic insulin clearance is only slightly affected (13). Hence, the liver is the main organ responsible for clearance of exogenous and in particular endogenous insulin (10). Systemic insulin concentrations are thus excessively high in liver cirrhosis explained by decreased hepatic insulin clearance (14), and liver transplantation normalizes the hyperinsulinemia (15). Hepatic insulin clearance involves insulin binding to the insulin receptor on hepatocytes and subsequent insulin receptor endocytosis (16). The insulin receptor has two isoforms derived from alternative splicing: type A and type B, with type B being primarily expressed in human liver (17). The molecular regulation of intrahepatic insulin degradation remains to be fully uncovered. It is believed that part of the insulin is degraded by lysosomal proteolysis in the hepatocytes. Here, an insulin-degrading enzyme (IDE) was proposed to be implicated, but molecular inhibition studies have recently questioned a major role of IDE in hepatic insulin clearance (18). Thus, insulin degradation was normal when measured in vitro in liver homogenates from cirrhotic compared with healthy subjects (19). This observation suggests that the main mechanisms behind insulin clearance involve insulin binding and receptor internalization. In agreement, inactivating insulin receptor mutations are associated with massive hyperinsulinemia resulting from severely impaired insulin clearance (20).

Whole-body insulin clearance can be estimated with reasonable accuracy during exogenous insulin infusions, as

during the hyperinsulinemic-euglycemic clamp (10) or the insulin suppression test (21). Coinfusion of somatostatin (or analogs thereof) allows for precise estimation of clearance of the exogenously administered insulin by eliminating endogenous insulin secretion (21). For endogenous insulin, the ratio of C-peptide to insulin concentrations may provide an estimate of hepatic insulin clearance. The latter approach builds on the assumption of absent hepatic extraction of C-peptide (8,11) and performs best in steady-state conditions (such as fasting) (22). The ratio can also be used during nonsteady state by use of areas-under-the-curves of C-peptide and insulin, provided that the concentration curves have returned to basal (22).

Absolute hepatic insulin clearance has been found to increase with increasing insulin concentrations, keeping the fractional insulin extraction stable (8,9). However, high portal insulin concentrations have been suggested to be associated with decreased insulin clearance due to receptor saturation (23), which is supported by findings of decreased fractional clearance during supraphysiological insulin infusions (10) and after excessive stimulation of endogenous insulin secretion with clamping at high glucose (300 mg/dL) concentrations for 2 h (24). Whether insulin clearance is saturable during normal physiological conditions (i.e., after meal intake) has been widely debated. Modeling of C-peptide and insulin concentrations showed reduced fractional hepatic insulin extraction with increasing oral loads of glucose in some (25,26) but not all (27) studies, whereas hepatic vein sampling demonstrated increasing absolute (and stable fractional) extraction of insulin with increasing glucose loads (28). Recently, a study with hepatic vein sampling suggested that hepatic insulin clearance increases in parallel with the arrival and passage of secretory insulin pulses, thus dampening systemic insulin oscillations (29), a finding which would be incompatible with acute saturation of the clearance mechanism during physiological conditions.

MODULATION BY DIETARY CARBOHYDRATE AND ENERGY EXCESS

In our dietary study, 3 days of 75% excess caloric intake with 80E% carbohydrate significantly decreased clearance of exogenous insulin (Fig. 1*A*) (6). Fasting plasma insulin concentrations were increased by 60%, whereas only minor changes in plasma C-peptide concentrations were observed, thus supporting decreased clearance of endogenous insulin as well. Observations in other studies support that short-term carbohydrate overfeeding decreases insulin clearance. Thus, in lean healthy men, 7 days of 50–70% energy excess with 60–86E% carbohydrate increased fasting plasma insulin concentrations by 51–188% (30,31) and augmented plasma insulin during an oral glucose tolerance test twofold (32). From these studies, the absolute carbohydrate intake seems to play an important role in modification of insulin clearance, though clearance was not measured directly. One study reported that progressive overfeeding with a 67E% carbohydrate-rich diet, reaching +210% energy excess within 4 days, increased fasting plasma insulin concentration by 150%, while fasting C-peptide increased by only 50% (33), indicating that decreased insulin clearance was a major contributor to the hyperinsulinemia. As more direct evidence of reduced insulin clearance, 13 days of 62% caloric excess by supplementation of a carbohydrate-rich drink to the habitual diet of lean healthy men was associated with a 10% increase in plasma insulin concentrations during a hyperinsulinemic-euglycemic clamp (34). Finally, increasing energy intake in lean healthy men by adding a carbohydrate-rich liquid drink to their diet, resulting in 7% body weight gain within ~4 weeks, decreased insulin clearance under an oral glucose tolerance test, while insulin clearance was restored to baseline values when subjects shifted to a hypocaloric diet (35). Similarly, in the early phase of weight gain induced by prolonged overeating, insulin clearance rather than increased insulin secretion was the mediator of the hyperinsulinemia induced by the weight gain (36).

MODULATION BY DIETARY CARBOHYDRATE AND ENERGY RESTRICTION

After RYGB, clearance of exogenous insulin increased from 12.9 to 16.2 mL/min/kg within 1 week after surgery (7), while surgical reversal of the RYGB had the opposite effect (16.6 to 14.2 mL/min/kg) in our recent case report (37). The fasting C-peptide–to–insulin ratios were similarly increased and decreased in response to RYGB and the reversal surgery, respectively (7,37). Other studies showing lower plasma insulin concentrations during hyperinsulinemic clamps and increased fasting plasma C-peptide–to–insulin ratio (38–41) are consistent with these findings of increased clearance of exogenous and endogenous insulin as an early (days to weeks) consequence of RYGB. Interestingly, it has been shown that RYGB-induced weight loss changes the expression of the liver insulin receptor isoforms A and B (42), with potential consequences for insulin affinity/signaling and hence insulin clearance.

Studies of short-term very-low-calorie diets also showed increased insulin clearance (43) and declines in plasma insulin concentrations within 48 h (44), supporting that increased insulin clearance is related to energy restriction rather than RYGB itself (41). After RYGB, energy intake is indeed acutely reduced by at least 35–50%, as is the total carbohydrate intake, while acute changes in macronutrient composition have not been consistently reported (45). Interestingly, 47% energy restriction with only 10E% carbohydrate induced a greater lowering of plasma insulin concentration than similar energy restriction with 65% carbohydrate (44), again supporting a role for the absolute carbohydrate availability for insulin clearance.

HEPATIC FAT CONTENT AND INSULIN CLEARANCE

The clearance of insulin is an integral part of insulin's action on the liver, i.e., both rely on insulin binding to the insulin receptor. The short-term manipulation with energy

and carbohydrate availability discussed above was associated with concomitant opposite changes in insulin clearance and glucose production (Fig. 1), supporting a link between hepatic insulin clearance and hepatic insulin sensitivity. An inverse association between insulin clearance and basal as well as insulin-suppressed hepatic glucose production has also been demonstrated in cross-sectional studies (46).

Hepatic triacylglycerol (TG) accumulation is likely to be a common link, as hepatic TG content correlates negatively with both insulin clearance and hepatic insulin sensitivity (47). This association between hepatic fat and insulin clearance is further supported by in vitro observations in hepatocytes, in which TG accumulation acutely reduces insulin clearance (48). Accordingly, hepatic fat was shown to be the closest correlate to fasting plasma insulin concentration in 271 subjects without diabetes (49). Thus, when subjects matched on BMI were divided in accordance to hepatic TG, fasting plasma insulin concentration was twofold higher in the group with high hepatic TG content (50). Similarly, insulin clearance differs markedly between metabolically healthy and unhealthy obese subjects of comparable BMI (51). Also, obese women with polycystic ovarian syndrome, known to have a high prevalence of hepatic steatosis, have 43% reduced insulin clearance when compared with BMI-matched women (52), and insulin clearance increases with dietary energy restriction (53).

In summary, total adiposity is not the primary determinant of insulin clearance, which is rather related to hepatic TG content (54). Studying the pathogenesis of hyperinsulinemia in obesity thus requires estimation of hepatic versus subcutaneous fat, which could explain some of the divergent results regarding the relative importance of insulin clearance versus insulin hypersecretion in obesity-related hyperinsulinemia (51,55). Also, ethnicity (56) and genetics (57) may contribute to the relative contribution of insulin clearance in the pathogenesis of hyperinsulinemia.

ENERGY AND CARBOHYDRATE EXCESS—HEPATIC TG AS THE LINK TO INSULIN CLEARANCE

Excess energy and carbohydrate intake versus restriction would be expected to have opposing effects on hepatic TG content. Increased monosaccharide availability combined with carbohydrate-induced postprandial insulin hypersecretion promote hepatic de novo lipogenesis (58,59), even at eucaloric conditions (60), which thereby could lead to increased hepatic TG content. The fasting plasma TG concentration increased substantially (1.7 ± 0.2 μmol/L) in healthy subjects after 3 days excess carbohydrate intake, while being reduced by 40% after the similarly hypercaloric high-fat diet with only 10E% carbohydrate (6). Likewise, fasting plasma TG was elevated 1.1- to 10-fold in other dietary studies with short-term overfeeding of carbohydrate-rich diets (30,32). A close and negative association was observed between the diet-induced changes in fasting plasma TG concentration and the changes in insulin clearance in the dietary study (Fig. 2). With fasting plasma VLDL-TG concentration reflecting hepatic TG content as

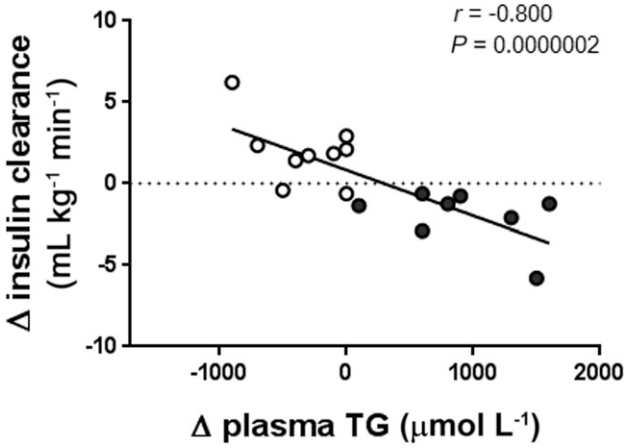

Figure 2—Scatter plot illustrating the association between the change in fasting plasma TG concentration and the change in insulin clearance after 3 days HIGH-CHO (black circles) and HIGH-FAT (white circles) dietary interventions compared with CON. CON: eucaloric diet with 65E% carbohydrate. HIGH-CHO: 3-day hypercaloric diet with 80E% carbohydrate and 11E% fat. HIGH-FAT: 3-day hypercaloric diet with 79E% fat and 10E% carbohydrate. Pearson correlation analysis was applied. Data from Lundsgaard et al. (6).

previously reported (61), these results suggest that accumulation of hepatic TG upon hypercaloric high carbohydrate intake is directly linked to decreased insulin clearance and vice versa during high fat/low carbohydrate intake.

The decreased insulin clearance after dietary carbohydrate excess may be mechanistically linked to the glycoprotein carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1). CEACAM1 promotes internalization of the insulin-receptor complex (62) by insulin receptor tyrosine kinase–induced phosphorylation of CEACAM1 (63), which subsequently forms part of a protein complex mediating insulin receptor endocytosis (64). Hence, CEACAM1 knockout markedly reduces insulin clearance in mice (65), while CEACAM1 reconstitution restores insulin clearance and reverses the insulin-resistant phenotype (66). Interestingly, CEACAM1 can also be associated with the enzyme fatty acid synthase (FAS), which is central to de novo lipogenesis; thus, in hyperinsulinemic *ob/ob* mice that have elevated FAS activity, insulin failed to induce CEACAM1 phosphorylation (67). The resulting lower CEACAM1 activity might lead to reduced insulin receptor endocytosis in conditions with high de novo lipogenesis. Furthermore, fatty acid (FA) products of de novo lipogenesis are shown to be ligands for peroxisome proliferator–activated receptor-α (PPARα) (68), with PPARα activation reducing CEACAM1 expression (69). CEACAM1 could therefore be a candidate hepatic molecule linking insulin clearance and carbohydrate-induced de novo lipogenesis–derived TG accumulation. In support of this, hepatic CEACAM1 protein expression was recently found to be reduced in obese compared with lean subjects (70) and in subjects with fatty liver disease (71).

HEPATIC FAT AFTER ENERGY AND CARBOHYDRATE RESTRICTION

Energy restriction introduced by RYGB has been demonstrated to reduce hepatic fat within 1 week and before major body weight loss (72). Also, 1 week of energy restriction by diet alone resulted in 30% reduction in hepatic TG content and 50% reduction in fasting plasma TG, concomitantly with 38% reduction in fasting plasma insulin, with unchanged C-peptide concentrations (73). Of note, as little as 48 h of energy restriction with low (10E%) compared with high (65E%) carbohydrate diet induced a threefold greater decrease in hepatic TG content, concomitant with a greater decrease in plasma insulin concentration and fasting glucose production (44). Together these findings suggest that energy restriction, and in particular restriction of total carbohydrate availability, acutely decreases hepatic TG content independent of weight loss and that this appears to be associated with a simultaneous increase in insulin clearance. Accordingly, it was recently shown that carbohydrate restriction under eucaloric conditions reduced hepatic TG content over 2 weeks and that the change was evident within 3 days, concomitantly with lowering of fasting plasma TG and insulin concentrations in obese subjects with fatty liver disease (74). This implies that changes in absolute carbohydrate intake can impose on liver fat content and regulation of plasma insulin concentrations independent of changes in energy availability. To this end, pharmacological lowering of hepatic TG content by rosiglitazone, which acts as a ligand of PPARγ receptors, is associated with a concomitant 20% increase in insulin clearance when administered to patients with T2D for 16 weeks, notably without weight loss (75).

A POTENTIAL ROLE OF HEPATIC GLYCOGEN OR GLUCOSE AVAILABILITY

Another potential link between carbohydrate intake and insulin clearance could be glycogen stores (or simply glucose availability) in the hepatocytes. The effect of hepatic glycogen on insulin clearance has not been directly investigated but may be of interest because acute prolonged exercise immediately increases insulin clearance in both lean and obese subjects (76), which is not related to altered exercise-induced peripheral insulin clearance (77). It could be speculated that this may relate to decreased hepatic glycogen content, as acute exercise of up to 2 h duration does not decrease hepatic TG content in healthy subjects (78).

THE ROLE OF THE FATTY ACIDS

In abdominal obesity, increased lipolytic activity of intra-abdominal adipose tissue could result in increased portal FA levels (79). It has therefore been speculated whether increased prehepatic FA availability plays a role in hepatic insulin metabolism. Associations between high FA availability and reduced insulin clearance have been suggested from lipid infusion studies in dogs (80) and in vitro studies in hepatocytes (81), although others have not been able to confirm this relationship in humans (47). An acute regulation of insulin clearance by FA delivery to the liver seems unlikely, as indicated by several lines of evidence. First, fasting plasma FA concentrations are substantially decreased (−68%) during carbohydrate overfeeding (6), proving that increased FA provision to the liver is not involved in the onset of decreased insulin clearance. Similarly, short-term energy restriction by RYGB or diet increases fasting plasma FA concentrations while simultaneously increasing insulin clearance (7,38,39,41). To this end, clamp insulin clearance measured directly across the hepatic artery and vein was unaltered after 8 h infusion of a TG emulsion (82). When glucose was then coinfused with a TG emulsion for 3 h, raising plasma glucose to 11 mmol/L, insulin clearance was impaired (83).

CONCLUDING REMARKS

Insulin clearance in the liver is a dynamic process that can be modified within days under conditions of changing energy and particularly carbohydrate intake and notably before major changes in basal insulin secretion. Early increases in insulin clearance after reduced energy intake (and thus reduced carbohydrate availability) are likely to be associated with metabolic adaptations in the liver similar to those observed during fasting, i.e., increased lipolysis and FA oxidation, resulting in lower TG accumulation. Interestingly, it has been shown that restriction of dietary carbohydrate rather than energy restriction per se initiates the metabolic response to fasting (84). Conversely, decreased insulin clearance is seen as an early response to carbohydrate overfeeding, which is potentially caused by concomitant increases in hepatic TG accumulation.

In the initial development of systemic hyperinsulinemia, reduced hepatic insulin clearance is therefore an important contributor, while insulin hypersecretion may contribute at later stages. Notably, hepatic insulin clearance is associated with hepatic rather than peripheral insulin sensitivity. In fact, it seems that the early impairments in insulin clearance precede the onset of peripheral changes in insulin action. Initially, increased systemic insulin concentrations induced by lower insulin clearance could serve to enhance peripheral glucose disposal, when hepatic substrate (carbohydrate) excess is present. This was clearly demonstrated in the dietary study where the carbohydrate-rich diet decreased hepatic insulin sensitivity and insulin clearance, concomitantly with 41% enhancement of peripheral glucose disposal (6). Also after energy restriction, a rapid improvement in hepatic insulin action and insulin clearance typically precedes the changes in peripheral insulin sensitivity, where improvements are not observed until substantial weight loss has been obtained (7,44,73). Thus, early changes in hepatic insulin clearance act to regulate systemic insulin availability and thereby affect the response of the peripheral tissues. In this context, it is of interest that 40 h of moderate hyperinsulinemia induces peripheral insulin resistance in healthy lean subjects (85) and that a low first-pass hepatic insulin extraction seems to determine peripheral insulin resistance in dogs (12).

In conclusion, hepatic insulin clearance is a major regulator of systemic insulin concentrations in the early response to altered energy and carbohydrate intake. While acknowledging the diversity of phenotypes in obesity and T2D including body composition, ethnicity, and genetics with additional contributions from a plethora of organ (e.g., β-cell) dysregulations in the development of overt T2D, our findings and the summarized evidence point to reduced hepatic insulin clearance as the initial culprit in the development of hyperinsulinemia. The causal link between hepatic carbohydrate availability, TG accumulation, and insulin clearance awaits further studies of potential mechanisms at the level of insulin receptor binding, internalization, and/or downstream intrahepatocellular signaling.

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