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1 Contents

List of abbreviations

ADA	American Diabetes Association
AIR	Acute Insulin Response
AUC	Area Under the Curve
BIGTT	Beta Cell Function Insulin Sensitivity Glucose Tolerance Test
BMI	Body Mass Index
CP	C-Peptide
CV	Coefficients Of Variation
EGP	Endogenous Glucose Production
G	Glucose
GLP-1	Glucagon-Like Peptide 1
H	Hour(S)
HbA1c	Glycated Haemoglobin
HC	Hyperglycaemic Clamp
HOMA	Homeostasis Model Assessment
I	Insulin
iAUC	Incremental Area Under the Curve
IFG	Impaired Fasting Glucose
IGI	Insulinogenic Index
IGT	Impaired Glucose Tolerance
IQR	Interquartile Range
IVGTT	Intravenous Glucose Tolerance Tests
MICE	Multivariate Chain Equation Imputation
NGT	Normal Glucose Tolerance
OGTT	Oral Glucose Tolerance Test
T2D	Type 2 Diabetes Mellitus
TUEF	Tübingen Family Study
WHO	World Health Organisation

1. Introduction

1.1 Definition, Epidemiology and Prevalence of Type 2 Diabetes

Diabetes mellitus is a complex multifactorial disorder characterized by elevated levels of plasma glucose due to impaired insulin secretion from pancreatic beta cells and insulin resistance in target tissues, causing numerous complications that may decrease the quality of life and life expectancy (Kawamori 2017).

The gradual and subtle progression of the disease poses challenges in establishing a clinically unambiguous diagnosis. This is also clear when looking at the diagnostic criteria for prediabetes from the American Diabetes Association (ADA) and the World Health Organization (American Diabetes Association 2021; World Health Organization and International Diabetes Federation 2006).

The ADA defines prediabetes as a disturbed fasting glucose level of 5.6 to 6.9 mmol/L, while the WHO only considers glucose values of 6.1 to 6.9 mmol/L as impaired fasting glucose and therefore prediabetes. The other diagnostic criteria for prediabetes and manifest type 2 diabetes mellitus are shown in the following table (Table 1) and are uniform.

Table 1: ADA and WHO definitions of prediabetes and diabetes mellitus. Plasma glucose concentration

		World Health Organisation (WHO)	**American Diabetes Association (ADA)**
Prediabetes	Impaired fasting glucose (IFG)	6,1- 6,9 mmol/L (110 mg/dl -125 mg/dL)	5,6-6,9 mmol/L (100 mg/dL-125 mg/dL)
	Impaired glucose tolerance (IGT)	≥7,8 and <11,1 mmol/l (140 mg/dL-199 mg/dL)	
	HbA1c	5,7-6,4%	

Diabetes Mellitus	2-h-OGTT	≥11,1 mmol/L (≥200 mg/dL)
	HbA1c	≥ 6,5% (48 mmol/mol)
	Random plasma glucose	≥11.1 mmol/L (≥200 mg/dL)

Type 2 diabetes is diagnosed when at least one of three criteria is met: a glycated haemoglobin (HbA1c) level of ≥ 6.5%, a fasting plasma glucose level of ≥ 126 mg/dL, or a 2-hour plasma glucose level of ≥ 200 mg/dL during an oral glucose tolerance test with 75 g of glucose.

While the hormone insulin has been the focus of diabetes development due to its established connection with glucose concentration in the blood, recent research shows that other factors also contribute to hyperglycaemia. In addition to insulin, incretin is another group of hormones that plays a significant role in regulating blood sugar levels. Incretin is a glucose-lowering group of hormones that was isolated from the intestine and named for its ability to regulate various metabolic processes, including insulin secretion, glucose concentrations, lipid metabolism, gut motility, appetite, and body weight. Glucagon-like peptide 1 (GLP-1) is a key incretin peptide that provides a scientific basis for using incretin-based therapies in the treatment of type 2 diabetes (Nauck and Meier 2018). Incretin deficiency and resistance also have a significant impact on diabetes development and disease progression (Holst and Gromada 2004; Nauck and Meier 2018).

According to statistics from the World Health Organization, more than 422 million adults worldwide were suffering from diabetes mellitus, with 90% of them having type 2 diabetes mellitus (Zheng, Ley, and Hu 2018). The International Diabetes Federation prognosticates that this burden will increase to 642 million by 2040 (Ogurtsova et al. 2017).

Furthermore, the global costs of diabetes and its consequences are enormous and constantly increase. According to the latest forecast, the full global costs of diabetes in adults through the year 2030 will increase to $2.25 trillion from $1.32 trillion in 2015 (Bommer et al. 2018, 203).

In light of the rapid growth of diabetes and its impact on human health, there is a pressing need to personalize and optimize treatment strategies while predicting and preventing complications. However, it is important to note that diabetes is a heterogeneous disease, characterized by diverse clinical manifestations and disease trajectories among individuals. Relying solely on one metabolite, such as glucose, for diagnosis poses challenges in accurately identifying patients who may benefit from intensive treatment or are at a higher risk of complications necessitating intensive prophylaxis and care.

To address this issue, a recently proposed subclassification of T2D takes into account, in addition to 3 other variables, insulin resistance and insulin sensitivity to extend the classical glucose-based definition (Ahlqvist et al. 2018). Clustering approach provides a more comprehensive view of the diabetes spectrum and allows for better predictions of potential complications by using reliable measures of insulin resistance and beta cell function.

In addition to this, prediabetes, an intermediary state of hyperglycaemia preceding T2D, is predominantly. This lack of detection and awareness further contributes to the complexity of understanding the different pathways leading to the development of metabolic disorders. As an approach to identify distinct groups of metabolisms, novel prediabetes subphenotypes have been proposed (Wagner et al. 2021). They are aimed to improve the identification of individuals at risk of developing diabetes and complications, individualize the approach to prevention, and reflect the key pathological features underlying different fates of metabolic complications (Wagner et al. 2021). As well as in assigning persons with diabetes to diabetes clusters, insulin resistance and insulin secretion indices are key components of this subclassification.

1.2 Insulin secretion

Insulin, a peptide hormone synthesized by beta cells within the pancreatic islets of Langerhans, plays a critical role in the regulation of glucose metabolism, ensuring the maintenance of glucose homeostasis in the human body. It serves as a key player in the intricate network of hormonal control, facilitating the uptake, utilization, and storage of glucose by various tissues, primarily the liver, adipose

tissue, and skeletal muscles. The insulin response to a stimulus is pulsatile, reflecting the coordinated secretory bursts of millions of beta cells (Satin et al. 2015). It is a complicated process involving a series of events in beta cells that conduct a fusion of secretory granules with the plasma membrane. Insulin secretion is primarily triggered by glucose, but other nutrients such as amino acids and free fatty acids can also enhance glucose-dependent insulin secretion (Fu, Gilbert, and Liu 2013). Additionally, GLP-1, a potent incretin hormone whose analogues are used in clinical practice, is able to augment insulin release in a glucose-dependent manner by acting on a G-protein–coupled receptor isoform (Wagner et al. 2014). Hence, the beta cell acts as a metabolic centre in the body, linking nutrient metabolism and the endocrine system.

Intravenous glucose injection promotes insulin secretion in the pancreas, which is considered as biphasic (Pfeifer, Halter, and Porte 1981). The glucose bolus triggers rapid release of insulin (“first-phase”) lasting approximately 10 minutes, followed by a slower "second-phase" of insulin secretion that appears 10 minutes after glucose administration and persists throughout the entire period of hyperglycaemia (DeFronzo, Tobin, and Andres 1979).

1.3 Insulin resistance

Insulin sensitivity refers to the ability of target tissues to take up glucose from the bloodstream in response to insulin. When the body is insulin sensitive, glucose uptake occurs at normal insulin concentrations. However, if higher insulin concentrations are needed to transport glucose to target tissues, a condition known as insulin resistance develops, leading to hyperglycaemia and reflex hyperinsulinemia (Lorenzo et al. 2010).

The effectiveness of insulin in regulating glucose metabolism depends on the sensitivity of effector cells. Although insulin resistance can occur in various effectors, it is most commonly associated with impaired glucose metabolism (Ferrannini and Mari 1998). Insulin resistance is a widespread phenomenon in both physiology and pathophysiology. For instance, pregnancy and puberty are examples of physiological disruptions of insulin action that limit glucose removal,

while obesity and diabetes are diseases that are often associated with impaired insulin sensitivity (Ferrannini and Mari 1998).

Hyperglycaemia, which defines T2D, is largely due to inadequate action insulin. When an elevated level of circulating insulin is required to accomplish the complex glucose-lowering response, the person is considered to be insulin resistant. On the other hand, hyperinsulinemia is an effective compensatory mechanism that preserves insulin action in mild to moderate insulin resistance. Several causative factors contributing to insulin resistance have been identified and studied (Petersen and Shulman 2018). Thus, prolonged fasting and stress contribute to insulin resistance, which limits glucose oxidation, promoting protein sparing (Soeters and Soeters 2012). High levels of sex steroids as well as high levels of growth hormone are responsible for decreased insulin sensitivity during puberty (Dahlgren 2006). Insulin resistance during pregnancy due to high placental lactogen levels and circulating insulin-like growth factor-1 maintains stable glucose levels and may increase foetal nutrient availability, possibly explaining the increased risk of foetal overgrowth (Dahlgren 2006).

It is considered that pathophysiological process of insulin resistance in most cases manifests itself at the cellular level through receptor- and post-receptor defects in insulin signalling. Possible mechanisms include genetic polymorphism, suppression or deficiency of insulin receptor tyrosine phosphorylation, insulin-responsive substrates proteins, or phosphatidylinositol protein 3-kinase, or may involve abnormalities in glucose transporter proteins function (Wilcox 2005).

1.4 Diabetes and prediabetes subphenotypes

Through extensive research conducted on diabetes, it has become evident that the disease exhibits remarkable heterogeneity. Gold-standard tests that evaluate beta cell function and insulin resistance have identified multiple patterns of insulin response, highlighting different subtypes of diabetes (Druet et al. 2006; Wood et al. 2017; Knebel et al. 2016). This demonstrates that the current diabetes definition, which is solely based on glucose, is insufficient in differentiating between mild and aggressive disease courses. Insulin resistance as well as inability of insulin-producing cells to keep up with insulin demand, or

a combination of these factors, plays a crucial role in the onset and progression of the disease.

In 2018 the novel clusters of diabetes (Ahlqvist et al. 2018) have changed the paradigm of diabetes studies and clearly demonstrated the different trajectories of diabetes progression. Several investigations have since been performed to allocate diverse diabetes subphenotypes based on insulin secretion and insulin sensitivity assessment (Zaharia et al. 2019; Sarría-Santamera et al. 2020; Kahkoska et al. 2020). Moreover, subphenotypes in prediabetes cohort demonstrated existence of pathophysiological heterogeneity long time before diabetes onset and stressed the necessity of subclassification before diabetes diagnosis (Wagner et al. 2021).

Early clustering in diabetes mellitus, as well as in prediabetes, using indices of insulin sensitivity and beta cell capacity, clearly identifies individuals at risk for complications and their rapid progression, opens new perspectives for prevention and care.

1.5 Assessment of beta cell function

New clustering approaches for subjects with and without diabetes have the potential to lead to a more individualized management of patients with diabetes (Ahlqvist et al. 2018; Wagner et al. 2021).

Accurate assessment of insulin sensitivity and secretion is critical to identify subphenotypes of prediabetes and type 2 diabetes (Ahlqvist et al. 2018; Wagner et al. 2021).

Insulin secretion and sensitivity are linked by a negative feedback loop in which beta cells compensate for changes in whole-body insulin sensitivity by proportionally and reciprocally increasing insulin secretion (Stumvoll and Gerich 2001).

Several methods are available for the estimation of insulin secretion and insulin resistance. Insulin sensitivity and secretion can be accurately estimated using hyperglycaemic and hyperinsulinemic-euglycemic clamps or intravenous glucose tolerance tests (page 13-14), which are assumed as the "gold standards" for these measurements (DeFronzo, Tobin, and Andres 1979; Bergman, Finegood,

and Ader 1985). However, in the IVGTT after the initial peak, glucose in the blood falls rapidly, and second-phase secretion is, in contrast to the hyperglycaemic clamp, not stable(Ferrannini and Mari 2019). Thus, the assessment of first-phase secretion is very similar between the hyperglycaemic clamp and the IVGTT, whereas second-phase secretion on the IVGTT occurs at variable glucose and is thus less representative.

In addition, these measures may help predict diabetes in non-diabetic subjects (Pratley and Weyer 2001, Lillioja et al. 1993). Nevertheless, the invasive, cost-consuming and labour-intensive procedures are not functional in everyday clinical practice.

Several indices such as C-peptide/ glucose ratio or Insulinogenic index 120 min have been proposed to evaluate insulin secretion on more easily measured values obtained after an oral glucose tolerance test (OGTT) or in the fasting state (den Biggelaar et al. 2017). Their use is affected by the degree of correlation with gold standard indicators, by their reproducibility (Hudak et al. 2021), and by their ability to predict diabetes incidence analogously to more sophisticated methods (Mari et al. 2001; Stumvoll et al. 2000; Otten, Ahrén, and Olsson 2014).

1.6 Research Hypothesis

The accurate assessment of insulin secretion is essential for understanding the heterogeneity of prediabetes and type 2 diabetes. While multiple surrogate markers for insulin sensitivity have been validated against gold-standard methods (Mari et al. 2001; Otten, Ahrén, and Olsson 2014), comprehensive assessments comparing insulin secretion indices remain limited. Although several measures for evaluating beta cells based on dynamic changes in insulin and glucose during OGTT have been analysed separately in numerous studies, most of these measures have not been directly compared, and there is still controversy regarding their validity (Hanson et al. 2000; Retnakaran et al. 2008; Tura, Kautzky-Willer, and Pacini 2006). Additionally, it is unclear whether any of these indices can provide reliable insights into GLP-1-stimulated insulin secretion, which could have potential clinical and research applications due to its crucial role in glucose homeostasis and the pathophysiology of diabetes.

Given these knowledge gaps, this study systematically evaluates multiple OGTT-derived insulin secretion indices in different glycemic groups. Based on existing literature, the following hypotheses were formulated:

- Among the OGTT-derived indices, only specific indices will demonstrate the strongest correlations with insulin secretion parameters measured by the hyperglycaemic clamp and IVGTT. These indices will be the most suitable for clinical and research applications, independent of glucose tolerance status.
- Certain OGTT-derived indices will be strongly associated with GLP-1-stimulated insulin secretion, as measured during the hyperglycemic clamp. These indices will be the most suitable for assessing GLP-1-related beta cell function.

2 Materials and Methods

2.1 Participants

The 392 participants enclosed in the current investigation were part of the Tübingen Family Study (TUEF). They were recruited between 2003 and 2018 based on an increased risk of developing type 2 diabetes (previous gestational diabetes, overweight, or positive family history).

The data from two well phenotyped cohorts was examined. The largest group of 316 subjects (183 females and 133 males) had intravenous glucose tolerance tests and oral glucose tolerance tests. Another cohort of 76 participants (43 females and 33 males) took part in hyperglycaemic clamps and oral glucose tolerance tests.

Informed written consent for the studies was obtained from all participants. The studies adhered to the Declaration of Helsinki. The study protocols were approved by the Ethical Committee of the Medical Faculty of the University of Tübingen (422/2002).

The classification of participants into different stages of glucose tolerance was performed according to the revised World Health Organization criteria (Alberti and Zimmet 1998). In the IVGTT-cohort 209 subjects were classified as normal glucose tolerance (NGT), 91 - impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), and 16 as test-diagnosed type 2 diabetes. In this cohort of 91 subjects with IFG/IGT, 28 of them had isolated IFG (fasting plasma glucose 6.1–6.9 mmol/l), 40 - isolated IGT (2-h glucose value 7.8–11.0 mmol/l), 23 -both IFG and IGT. The Hyperglycaemic clamp group contains 49 NGT, 23 prediabetes (IFG and/or IGT) and 4 clamp-diagnosed T2D participants.

2.2 Hyperglycaemic clamp

A modified hyperglycaemic clamp with additional stages of GLP-1 and arginine stimulation was performed as proposed by Fritsche et al (Fritsche et al. 2000). During a single 3.5 hours session, the test evaluates first- and second-phase of

insulin secretion induced by intravenous glucose bolus, as well as insulin response to an incretin stimulus and a peak stimulated insulin secretion elicited by an arginine bolus. The clamp was initiated with a weight-adapted intravenous bolus of 20% glucose over 1 min and continued with an infusion of 20% glucose with periodic adjustments based on the negative feedback principle to maintain blood glucose at 180 mg/dl. In other words, the glucose level rises after the intravenous bolus injection and is maintained at the target level of 180 mg/dl by periodically adjusting the glucose infusion rate. GLP-1 was given as a bolus injection (4.5 pmol kg^{-1}) after 120 minutes, followed by a continuous infusion of 1.5 pmol $kg^{-1}min^{-1}$during the next 60 min. Serum samples for insulin, C-peptide, and glucose were drawn at -30, -15, 0, 2.5, 5, 7.5, 10, 15, 30, 60, 90, 120, 140, 160, 170, 180 min. Insulin secretion after bolus administration of arginine at the end of the clamp was not analysed in this work.

The first-phase of insulin release, reflecting the early peak of insulin secreted by pancreatic beta cells in response to glucose stimulation, was calculated as the sum of insulin or C-peptide measurements at 2.5, 5, 7.5, and 10 minutes. The second-phase of glucose-stimulated insulin secretion was emanated as means of insulin or C-peptide at 80, 100 and 120 min. GLP-1-stimulated insulin (C-peptide) secretion at 10 mM glucose was evaluated as a mean of 160, 170 and 180 min.

2.3 Intravenous glucose tolerance test

The intravenous glucose tolerance test (IVGTT) is used to accurately assess the first-phase of insulin release (Darden et al. 2020). Measurements of glucose and insulin dynamics during the first 10 minutes (first-phase) reflect the ability of beta cells to produce insulin and the effectiveness of its action. Abnormal results could be recorded before the onset of diabetes, but due to the time-consuming and expensive procedure, the test is usually used only for scientific purposes. Compared to OGTT, the glucose sample does not have to pass through the digestive system, which makes the assay more sensitive.

During intravenous glucose tolerance test, an intravenous bolus of glucose after baseline blood draw was given at minute 0 (0.3 g/kg body weight in a 20%

solution). At minutes 0, 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 min was sampled blood for measure insulin, C-peptide (CP) and plasma glucose concentration (Staiger et al. 2007). Using the trapezoidal method was determined the incremental area under the curve ($iAUC_{0-10}$) for insulin and C-peptide.

2.4 Oral glucose tolerance test

The oral glucose tolerance test (OGTT) is used both in clinical practice and in research to assess glucose tolerance. OGTT can be used for surrogate measurements of insulin sensitivity and insulin response to enteral glucose and is widely used to assess beta cell dysfunction in obesity, prediabetes, and type 2 diabetes (Chen, Aguirre, and Hannon 2018).

The standardized glucose solution used (Accu Check Dextrose, Roche) contains 75g of anhydrous glucose. All 392 persons participated in the OGTT, taking 75 g of glucose in a volume of 300 ml after an overnight fast. After basal blood sampling through a peripheral venous cannula (Abbocath 20G), the glucose solution was drunk within 3-5 minutes after fasting for at least 8 hours. Blood samples were taken after 15, 30, 60, 90, 120 and 150 minutes. The blood samples were immediately stored on ice and sent to the central laboratory of the University Hospital in Tübingen. Insulin secretion indices were calculated from these OGTTs.

2.5 Blood Collection and Measures

Blood was taken through a venepuncture cannula during the preliminary examination and through a peripheral indwelling cannula in the crook of the elbow or on the forearm during the tests.

Rinsing with 2 ml NaCl 0.9% after each blood collection ensured that the indwelling cannula was used for blood collection and occlusion by coagulated blood was avoided. Before each blood sampling, 2 ml of the blood was taken separately to prevent falsification of the values.

The blood samples were immediately stored on ice and processed in the central laboratory of the university hospital.

C-peptide and insulin concentrations were determined using chemiluminescent methods on an ADVIA Centaur XPT analyser (Siemens Healthineers, Eschborn, Germany). Glucose concentrations were measured using a hexokinase method on an ADVIA clinical chemistry XPT analyser (Siemens Healthineers). The limits of quantification (LoQ) of the insulin and C-peptide assays are 1 pmol/l and 16 pmol/l, respectively. Using quality control samples for determination of assay imprecision, typical coefficients of variation (CV) were obtained: 4.6% (target concentration: 136 pmol/l) and 5.2% (597 pmol/l) using the insulin assay and 5.8% (337 pmol/l) and 6.2% (1470 pmol/l) using the C-peptide assay.

2.6 Multivariate Imputation

For missingness in the dataset, which were less than 2.6% and completely random, a method called multivariate imputation by chained equations (Azur et al. 2011) was performed. Multivariate chain equation imputation (MICE) is a commonly used method for dealing with missing data and unlike to single imputations, the creation of multiple imputations takes into account the statistical uncertainty of imputations (Azur et al. 2011). It is more robust than single imputations and can handle variables of different types due to its flexible nature. The method of chained equations involves filling in the missing values for each variable separately, using information from other variables in the dataset. This process is repeated multiple times to create several complete datasets. The imputations are then combined to obtain parameter estimates and standard errors, which take into account the uncertainty introduced by the missing data. Studies have shown that multiple imputations of missing data, even when the percentage of missing data about 5%, does not lead to biases or loss of power in statistical analyses(Azur et al. 2011; Graham 2009).

2.7 Insulin secretion indices

One of the most frequently used indices of beta cell function is the insulinogenic index (IGI), an index of insulin secretion suggested in 1967 (Seltzer et al. 1967). It was computed as $(I_{30} - I_0)/(G_{30} - G_0)$ and $(I_{120} - I_0)/(G_{120} - G_0)$, with I_n, G_n, plasma

concentrations at the *n*th minute for plasma glucose and insulin respectively (Seltzer et al. 1967).

A novel method for the assessment of insulin secretion was proposed and validated in 2007 by a group of scientists from Denmark (Hansen et al. 2007). The beta cell function insulin sensitivity glucose tolerance test (BIGTT) method is an estimation of acute insulin response (AIR) derived from the following equation: $BIGTT\text{-}AIR_{0\text{-}30\text{-}120} = \exp[8.20 + (0.00178 \times I_0) + (0.00168 \times I_{30}) - (0.000383 \times I_{120}) - (0.314 \times G_0) - (0.109 \times G_{30}) + (0.0781 \times G_{120}) + (0.180 \times sex) + (0.032 \times BMI)]$, $BIGTT\text{-}AIR_{0\text{-}60\text{-}120} = \exp[8.20 + (0.00178 \times I_0) + (0.00168 \times I_{60}) - (0.000383 \times I_{120}) - (0.314 \times G_0) - (0.109 \times G_{60}) + (0.0781 \times G_{120}) + (0.180 \times sex) + (0.032 \times BMI)]$ (Hansen et al. 2007).

The ratio of the AUC for insulin and for C-peptide to AUC for glucose over a specified time frame was calculated by applying the trapezoid rule (AUC (I_{all}), AUC (CP_{all}), AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), AUC ($CP_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), AUC ($I_{0\text{-}120}$)/AUC ($G_{0\text{-}120}$), AUC ($CP_{0\text{-}120}$)/AUC ($G_{0\text{-}120}$)) (Retnakaran et al. 2008).

The HOMA-%B was calculated using the fasting plasma insulin and glucose concentration ($HOMA\text{-}\%B = (20 \times I_0)/(G_0 - 3.5)$) (Wallace, Levy, and Matthews 2004). Fasting insulin, C-peptide and plasma glucose quantities were used in the homeostasis model assessment to generate estimates of beta cell function (HOMA2%B) (Wallace, Levy, and Matthews 2004).

Further indices of insulin secretion used in this analysis were as follows: the insulin/glucose ratio derived as I_0/G_0 and I_{120}/G_{120}, C-peptide/ glucose ratio CP_0/G_0 and CP_{120}/G_{120} , insulin and C-peptide ratio at minute 30 (I_{30}/I_0, CP_{30}/CP_0) and at minute 120 (I_{120}/I_0, CP_{120}/CP_0), delta insulin at 30 min $\Delta I_{30} = I_{30} - I_0$, C-peptide and insulin at fasting state (CP_0, I_0), minute 30 (CP_{30}, I_{30}) and minute 120 (CP_{120}, I_{120}) (den Biggelaar et al. 2017); the corrected insulin response (CIR) = $I_{30}/(G_{30} \times (G_{30} - 3.89))$ and $I_{120}/(G_{120} \times (G_{120} - 3.89))$ (Hanson et al. 2000); first-phase Stumvoll = $1283 + 1.829 \times I_{30} - 138.7 \times G_{30} + 3.772 \times I_0$; and second-phase Stumvoll = $286 + 0.416 \times I_{30} - 25.94 \times G_{30} + 0.926 \times I_0$ (Stumvoll et al. 2000) ; C-peptide index (CPI) $CPI_{120} = 100 \times CP_{120}(ng/mL)/G_{120}(mg/dL)$, $CPI_0 = 100 \times CP_0(ng/mL)/G_0(mg/dL)$ ('OGTT 1h Serum C-Peptide to Plasma Glucose Concentration Ratio Is More Related to Beta Cell Function and Diabetes Mellitus',

n.d.); Kadowaki model = $(I_{30} - I_0)/G_{30}$ (Kadowaki et al. 1984); log transformed insulin at minute 0,30,60,90,120 ($\log(I_0)$, $\log(I_{30})$, $\log(I_{60})$, $\log(I_{90})$, $\log(I_{120})$) (Sun et al. 2013). All indices are summarised in the Table 2.

Table 2: Fasting- and OGTT-derived measures of beta cell function

Fasting/OGTT-based indices	Formula
$AUC(CP_{0-120})/AUC\ (G_{0-120})$	AUC for insulin, glucose and C-peptide were calculated with the trapezoid method
$AUC(CP_{0-30})/AUC(G_{0-30})$	
$AUC\ (CP_{0-60})/AUC\ (G_{0-60})$	
$AUC(CP_{all})$	
$AUC(I_{all})$	
$AUC(I_{0-120})/AUC(G_{0-120})$	
$AUC(I_{0-30})/AUC(G_{0-30})$	
$AUC(I_{0-60})/AUC(G_{0-60})$	
$BIGTT\text{-}AIR_{0-30-120}$	$\exp[8.20 + (0.00178 \times I_0) + (0.00168 \times I_{30}) - (0.000383 \times I_{120}) - (0.314 \times G_0) - (0.109 \times G_{30}) + (0.0781 \times G_{120}) + (0.180 \times \text{sex}) + (0.032 \times \text{BMI})]$
$BIGTT\text{-}AIR_{0-60-120}$	$\exp[8.20 + (0.00178 \times I_0) + (0.00168 \times I_{60}) - (0.000383 \times I_{120}) - (0.314 \times G_0) - (0.109 \times G_{60}) + (0.0781 \times G_{120}) + (0.180 \times \text{sex}) + (0.032 \times \text{BMI})]$
CIR_{120}	$I_{120} /(G_{120} \times (G_{120} - 3.89))$
CIR_{30}	$I_{30} /(G_{30} \times (G_{30} - 3.89))$
CIR_{60}	$I_{60} /(G_{60} \times (G_{60} - 3.89))$
CP_{120}/CP_0	CP_{120}/CP_0
CP_{30}/CP_0	CP_{30}/CP_0
CP_{60}/CP_0	CP_{60}/CP_0
CP_0	

CP_0/G_0	CP_0/G_0
CP_{120}	
CP_{120}/G_{120}	CP_{120}/G_{120}
CP_{30}	
CP_{30}/G_{30}	CP_{30}/G_{30}
CP_{60}	
CP_{60}/G_{60}	CP_{60}/G_{60}
HOMA-%B	$(20 \times I_0)/(G_0 - 3.5)$
HOMA2-%B(CP)	https://www.dtu.ox.ac.uk/homacalculator/
HOMA2-%B(I)	https://www.dtu.ox.ac.uk/homacalculator/
I_0	
I_0/G_0	I_0/G_0
I_{120}	
I_{120}/G_{120}	I_{120}/G_{120}
I_{30}	
ΔI_{30}	$I_{30} - I_0$
I_{30}/G_{30}	I_{30}/G_{30}
I_{60}	
ΔI_{60}	$I_{60} - I_0$
I_{60}/G_{60}	I_{60}/G_{60}
IGI_{120}	$(I_{120} - I_0)/(G_{120} - G_0)$
IGI_{30}	$(I_{30} - I_0)/(G_{30} - G_0)$
IGI_{60}	$(I_{60} - I_0)/(G_{60} - G_0)$
I_{120}/I_0	I_{120}/I_0
I_{30}/I_0	I_{30}/I_0
I_{60}/I_0	I_{60}/I_0

Kadowaki model	$(I_{30} - I_0)/G_{30}$
$\log(I_0)$	$\log(I_0)$
$\log(I_{120})$	$\log(I_{120})$
$\log(I_{30})$	$\log(I_{30})$
$\log(I_{60})$	$\log(I_{60})$
$\log(I_{90})$	$\log(I_{90})$
CPI_0	$100 \times CP_0(ng/mL)/G_0(mg/dL)$
CPI_{120}	$100 \times CP_{120}(ng/mL)/G_{120}(mg/dL)$
first-phase Stumvoll	$1283 + 1.829 \times I_{30} - 138.7 \times G_{30} + 3.772 \times I_0$
second-phase Stumvoll	$286 + 0.416 \times I_{30} - 25.94 \times G_{30} + 0.926 \times I_0$

2.8 Body-Mass-Index (BMI)

BMI describes the quotient of body weight in kilograms and the square of height in meters.

To determine the weight, the same scale was used for all measurements. 1 kg was subtracted from the weight of the clothed subject.
The BMI is a widely used measure for determining obesity and assessing the associated risk of developing secondary diseases such as type 2 diabetes mellitus and cardiovascular diseases (Huxley et al. 2010).
The WHO limits were used to assess the BMI (‘A Healthy Lifestyle - WHO Recommendations’, n.d.).

Table 3: Nutritional status

BMI	Nutritional status
Below 18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Pre-obesity
30.0–34.9	Obesity class I
35.0–39.9	Obesity class II
Above 40	Obesity class III

2.9 Statistical analysis

Statistical analyses were performed with R (software version 4.0.3), a powerful statistical computing tool for data analysis and plotting (R Core Team 2020). With the R system for statistical computing, the study can be reproduced using the R transcript file provided in the supplementary file, where the results of all data analysis steps are presented to readers.

Descriptive data are expressed as medians ± IQR. The insulin and C-peptide-derived insulin secretion indices were separately analysed. Due to the relatively long half-life of C-peptide (20–30 minutes), indexes based on C-peptide reflect only insulin secretion, while indexes based on insulin concentration, which half-life is only 3–5 minutes, reflect a combination of insulin secretion and insulin clearance (Wallace, Levy, and Matthews 2004; Marques, Fontaine, and Rogers 2004; Wahren and Larsson 2015).

The relationship between the different insulin secretion indices and reference gold-standard measures was determined using both Pearson's and Spearman's correlation coefficients. These tests were applied to a predefined set of indices in two independent cohorts (n=316 and n=76), ensuring reproducibility of results.

The Pearson correlation coefficient was discovered in 1846 by Bravais, and was firstly described by Karl Pearson in 1896 (Hauke and Kossowski, n.d.). An essential assumption of which is the normality of the investigated variables, which can only be true for quantitative variables (Fujita et al. 2009). The Pearson

correlation coefficient is an evaluation of the strength of a linear association between two such variables (Fujita et al. 2009).

On the other hand, Spearman's rank correlation coefficient, adopted from Pearson's correlation coefficient and described in 1904, is a non-parametric (no distribution) rank statistic approach that measures the strength of an association between two variables that cannot be quantified and use data transformation that replaces numeric values by their rank when sorting data. (Hauke and Kossowski, n.d.). It evaluates how well an arbitrary monotonic function can explain the association between two variables.

Both correlation approaches were used, and the best-performing indices were sorted in ascending order using Pearson's correlation coefficients reflecting linear relationships between variables and given alongside Spearman's correlation coefficients. Given that the study is designed as a validation analysis with a limited number of predefined tests, multiple testing correction was not applied. To ensure robust interpretation, only the strongest correlations ($r > 0.6$) were highlighted. Statistical significance was set at $p < 0.05$.

3 Results

Parts of the data presented here have been published (Katsiaryna Prystupa et al. 2022).

3.1 Subject Characteristics

At the beginning of the analysis, the anthropometric and glycaemic characteristics of test persons were determined. The clinical and biochemical characteristics of the study cohorts are outlined in Table 4.

A total of 392 subjects underwent OGTT. A group of 316 participants (183 females and 133 males) had oral glucose tolerance test as well as intravenous glucose tolerance tests, while another group of 76 subjects (43 females and 33 males) underwent hyperglycaemic clamps and oral glucose tolerance test.

Variables are reported as median and interquartile ranges. The gender f=female and m=male is given in absolute frequencies. The number of persons with impaired fasting glucose or/and impaired glucose tolerance as well as the number of subjects with diabetes type 2 is given in absolute and relative frequencies, percentage of variables is written in parenthesis.

Table 4: Clinical and biochemical characteristics of the study population

participated in OGTT and IVGTT	
n	316
Sex m/f	183/133
Age (median [IQR])	47.00 [37.00, 54.00]
BMI (median [IQR])	28.59 [25.11, 32.20]
NGT (%)	209 (66.1%)
IFG and/or IGT	91 (28.8%)
Type 2 diabetes	16 (5.1%)

participated in OGTT and hyperglycaemic clamp	
n	76
Sex m/f	33/43
Age (median [IQR])	36.00 [26.00, 46.25]
BMI (median [IQR])	24.48 [22.01, 26.94]
NGT	49 (64.5 %)
IFG and/or IGT	23 (30.2 %)
Type 2 diabetes	4 (5.3 %)

In the first group of participants, there is an excess of males compared to females (58 % against 42%). In the group of subjects who participated in OGTT and hyperglycaemic clamp the percentage of males is slightly lower than females (43% vs 57%).

The median age in the first group was 47 years with an interquartile range of 37 to 54 years. For the second group of persons, the age median is 36 with an interquartile range of 26 to 46.

The median BMI in the group of subjects who participated in OGTT and IVGTT is 28.59 kg/m^2, and 24.48 kg/m^2 in the other group indicating that most of the persons in the cohort are nonobese. The percentage of subjects with prediabetes such as impaired fasting glucose and/or impaired glucose tolerance is balanced in both groups, 28,8% in the group of persons who participated in OGTT and GST and 30,2% in another group.

3.2 Comparison of insulin secretion indices with dynamic insulin secretion measurements by IVGTT

The insulin secretion indices obtained from fasting or OGTT measurements were tested against IVGTT-obtained measurements (Insulin $iAUC_{0\text{-}10}$ and C-peptide $iAUC_{0\text{-}10}$) of beta cell capacity.

Most tested surrogate insulin secretion indices except for CP_{120}/CP_0 and I_{120}/I_0 correlated significantly with first-phase insulin and C-peptide-based IVGTT-values in the group of NGT.

Table 5: NGT-group (n=209). Correlation between fasting and OGTT-derived insulin secretion indices and first-phase insulin secretion measured with the IVGTT (* p<0.05; **p<0.01; ***p<0.001).

Indices	Insulin $iAUC_{0\text{-}10}$ Spearman	C-peptide $iAUC_{0\text{-}10}$ Spearman	Insulin $iAUC_{0\text{-}10}$ Pearson	C-peptide $iAUC_{0\text{-}10}$ Pearson
$AUC(CP_{0\text{-}120})/AUC(G_{0\text{-}120})$	0.428 ***	0.475 ***	0.392 ***	0.445 ***
$AUC(CP_{0\text{-}30})/AUC(G_{0\text{-}30})$	0.546 ***	0.572 ***	0.556 ***	0.58 ***
$AUC(CP_{0\text{-}60})/AUC(G_{0\text{-}60})$	0.403 ***	0.443 ***	0.364 ***	0.407 ***
$AUC(CP_{all})$	0.311 ***	0.296 ***	0.297 ***	0.3 ***
$AUC(I_{all})$	0.435 ***	0.355 ***	0.439 ***	0.373 ***
$AUC(I_{0\text{-}120})/AUC(G_{0\text{-}120})$	0.525 ***	0.462 ***	0.534 ***	0.48 ***
$AUC(I_{0\text{-}30})/AUC(G_{0\text{-}30})$	0.635 ***	0.576 ***	0.665 ***	0.604 ***
$AUC(I_{0\text{-}60})/AUC(G_{0\text{-}60})$	0.423 ***	0.367 ***	0.399 ***	0.379 ***
$BIGTT\text{-}AIR_{0\text{-}30\text{-}120}$	0.651 ***	0.596 ***	0.697 ***	0.619 ***
$BIGTT\text{-}AIR_{0\text{-}60\text{-}120}$	0.531 ***	0.5 ***	0.54 ***	0.534 ***
CIR_{120}	0.259 ***	0.255 ***	0.129	0.155 *
CIR_{30}	0.646 ***	0.642 ***	0.616 ***	0.616 ***
CIR_{60}	0.43 ***	0.447 ***	0.288 ***	0.341 ***
CP_{120}/CP_0	-0.109	-0.105	-0.13	-0.118

CP_{30}/CP_0	0.264 ***	0.301 ***	0.201 **	0.241 ***
CP_{60}/CP_0	-0.048	-0.02	-0.092	-0.07
CP_0	0.31 ***	0.278 ***	0.342 ***	0.323 ***
CP_0/G_0	0.314 ***	0.294 ***	0.355 ***	0.344 ***
CP_{120}	0.183 **	0.164 *	0.156 *	0.152 *
CP_{120}/G_{120}	0.203 **	0.225 **	0.179 **	0.21 **
CP_{30}	0.52 ***	0.523 ***	0.484 ***	0.496 ***
CP_{30}/G_{30}	0.57 ***	0.609 ***	0.555 ***	0.593 ***
CP_{60}	0.291 ***	0.273 ***	0.26 ***	0.264 ***
CP_{60}/G_{60}	0.402 ***	0.47 ***	0.334 ***	0.401 ***
HOMA-%B	0.439 ***	0.357 ***	0.483 ***	0.417 ***
HOMA2-%B(CP)	0.286 ***	0.284 ***	0.313 ***	0.32 ***
HOMA2-%B(I)	0.401 ***	0.333 ***	0.475 ***	0.404 ***
I_0	0.456 ***	0.356 ***	0.499 ***	0.402 ***
I_0/G_0	0.468 ***	0.375 ***	0.517 ***	0.425 ***
I_{120}	0.322 ***	0.254 ***	0.297 ***	0.228 ***
I_{120}/G_{120}	0.352 ***	0.296 ***	0.339 ***	0.28 ***
I_{30}	0.586 ***	0.519 ***	0.582 ***	0.524 ***
ΔI_{30}	0.579 ***	0.517 ***	0.567 ***	0.515 ***
I_{30}/G_{30}	0.645 ***	0.594 ***	0.682 ***	0.627 ***
I_{60}	0.326 ***	0.255 ***	0.316 ***	0.288 ***
ΔI_{60}	0.3 ***	0.235 ***	0.288 ***	0.267 ***
I_{60}/G_{60}	0.434 ***	0.395 ***	0.401 ***	0.393 ***
IGI_{120}	0.222 **	0.164 *	0.136	0.109
IGI_{30}	0.665 ***	0.655 ***	0.583 ***	0.594 ***
IGI_{60}	0.406 ***	0.423 ***	0.184 **	0.246 ***
I_{120}/I_0	-0.071	-0.065	-0.033	-0.022
I_{30}/I_0	0.255 ***	0.272 ***	0.193 **	0.242 ***
I_{60}/I_0	-0.078	-0.053	-0.109	-0.066
Kadowaki model	0.635 ***	0.588 ***	0.662 ***	0.613 ***
$\log(I_0)$	0.456 ***	0.356 ***	0.491 ***	0.398 ***
$\log(I_{120})$	0.322 ***	0.254 ***	0.325 ***	0.257 ***

log(I_{30})	0.586 ***	0.519 ***	0.572 ***	0.523 ***
log(I_{60})	0.326 ***	0.255 ***	0.305 ***	0.267 ***
log(I_{90})	0.204 **	0.133	0.209 **	0.132
CPI_0	0.314 ***	0.294 ***	0.355 ***	0.344 ***
CPI_{120}	0.203 **	0.225 **	0.179 **	0.21 **
first-phase Stumvoll	0.658 ***	0.615 ***	0.686 ***	0.631 ***
second-phase Stumvoll	0.561 ***	0.479 ***	0.574 ***	0.504 ***

In NGT, the strongest correlation with first-phase insulin and C-peptide by both Spearman's and Pearson's methods was found for CIR_{30}, followed by I_{30}/G_{30}, BIGTT-$AIR_{0\text{-}30\text{-}120}$, first-phase Stumvoll and AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$).

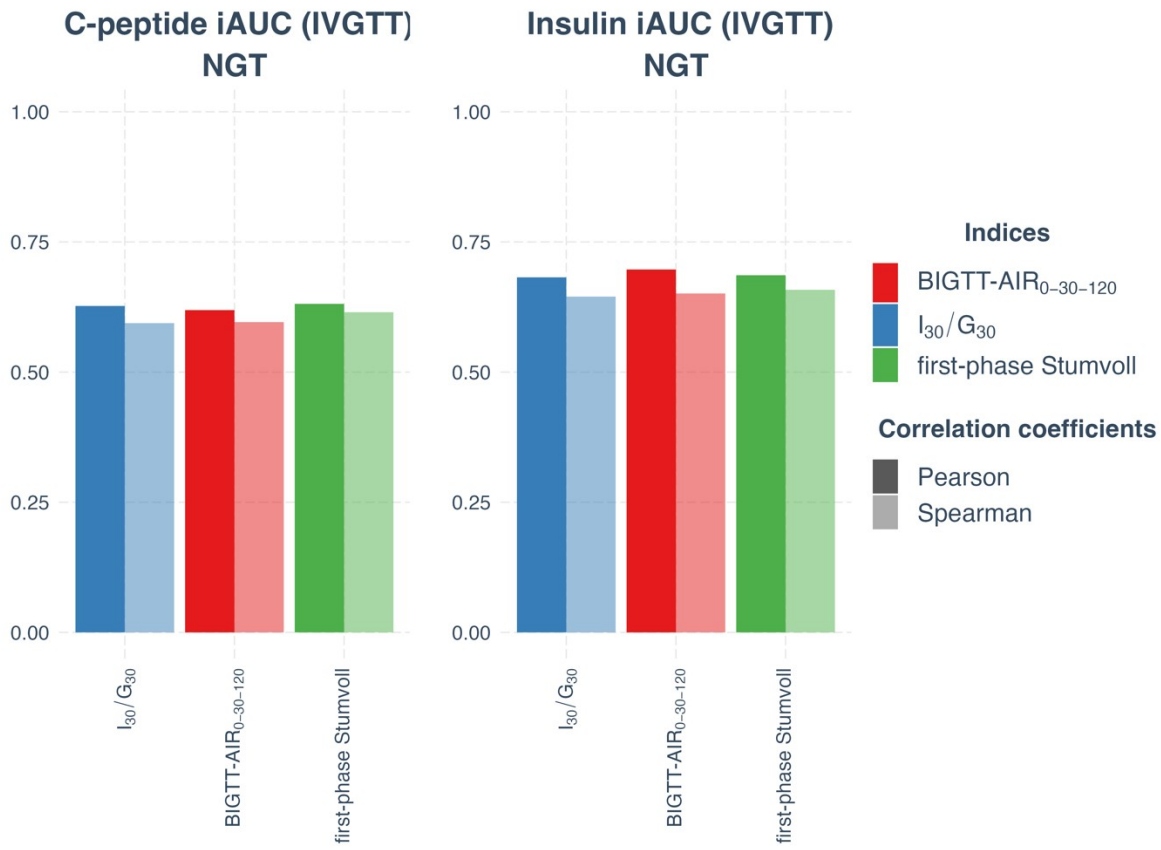


Figure 1: OGTT-indices with the highest Spearman's and Pearson's correlation coefficients in subset with NGT compared to first-phase IVGTT

At the same time, I determined the indices at the bottom of the list of correlations, with the lowest significant correlation coefficients. These indices in the NGT-group included CPI_{120}, CP_{120}/G_{120} and CP_{120} (Table 5).

In the prediabetes group with more than 90 participants, the same analysis was performed. Similarly to the NGT group, most of the surrogate indices correlate significantly with IVGTT-derived measurements of beta cell secretion in prediabetes group.

Table 6: IFG and/or IGT -group (n=91). Correlation between fasting and OGTT-derived insulin secretion indices and first-phase insulin secretion measured with the IVGTT (* p<0.05; **p<0.01; ***p<0.001).

Indices	Insulin $iAUC_{0-10}$ Spearman	C-peptide $iAUC_{0-10}$ Spearman	Insulin $iAUC_{0-10}$ Pearson	C-peptide $iAUC_{0-10}$ Pearson
$AUC(CP_{0-120})/AUC(G_{0-120})$	0.573 ***	0.551 ***	0.501 ***	0.513 ***
$AUC(CP_{0-30})/AUC(G_{0-30})$	0.597 ***	0.56 ***	0.555 ***	0.544 ***
$AUC(CP_{0-60})/AUC(G_{0-60})$	0.591 ***	0.564 ***	0.563 ***	0.56 ***
$AUC(CP_{all})$	0.453 ***	0.42 ***	0.426 ***	0.407 ***
$AUC(I_{all})$	0.48 ***	0.367 ***	0.355 ***	0.252 *
$AUC(I_{0-120})/AUC(G_{0-120})$	0.555 ***	0.45 ***	0.405 ***	0.31 **
$AUC(I_{0-30})/AUC(G_{0-30})$	0.634 ***	0.537 ***	0.513 ***	0.424 ***
$AUC(I_{0-60})/AUC(G_{0-60})$	0.542 ***	0.426 ***	0.465 ***	0.378 ***
$BIGTT\text{-}AIR_{0-30-120}$	0.483 ***	0.365 ***	0.411 ***	0.295 **
$BIGTT\text{-}AIR_{0-60-120}$	0.468 ***	0.347 ***	0.51 ***	0.424 ***
CIR_{120}	0.459 ***	0.433 ***	0.17	0.198
CIR_{30}	0.565 ***	0.496 ***	0.452 ***	0.416 ***
CIR_{60}	0.598 ***	0.518 ***	0.396 ***	0.382 ***
CP_{120}/CP_0	-0.128	-0.079	-0.169	-0.11
CP_{30}/CP_0	0.321 **	0.366 ***	0.274 **	0.303 **
CP_{60}/CP_0	0.112	0.183	0.102	0.156
CP_0	0.331 **	0.272 **	0.345 ***	0.294 **

CP_0/G_0	0.356 ***	0.293 **	0.367 ***	0.319 **
CP_{120}	0.277 **	0.249 *	0.213 *	0.206
CP_{120}/G_{120}	0.452 ***	0.423 ***	0.312 **	0.326 **
CP_{30}	0.614 ***	0.575 ***	0.578 ***	0.56 ***
CP_{30}/G_{30}	0.629 ***	0.613 ***	0.566 ***	0.576 ***
CP_{60}	0.485 ***	0.466 ***	0.513 ***	0.503 ***
CP_{60}/G_{60}	0.604 ***	0.593 ***	0.561 ***	0.578 ***
HOMA-%B	0.452 ***	0.309 **	0.421 ***	0.308 **
HOMA2-%B(CP)	0.382 ***	0.323 **	0.369 ***	0.333 **
HOMA2-%B(I)	0.421 ***	0.282 **	0.419 ***	0.308 **
I_0	0.418 ***	0.296 **	0.427 ***	0.326 **
I_0/G_0	0.438 ***	0.308 **	0.446 ***	0.343 ***
I_{120}	0.289 **	0.206	0.232 *	0.134
I_{120}/G_{120}	0.385 ***	0.308 **	0.272 **	0.19
I_{30}	0.63 ***	0.535 ***	0.49 ***	0.401 ***
ΔI_{30}	0.63 ***	0.539 ***	0.478 ***	0.397 ***
I_{30}/G_{30}	0.634 ***	0.548 ***	0.513 ***	0.435 ***
I_{60}	0.468 ***	0.372 ***	0.423 ***	0.355 ***
ΔI_{60}	0.46 ***	0.373 ***	0.413 ***	0.349 ***
I_{60}/G_{60}	0.562 ***	0.458 ***	0.473 ***	0.394 ***
IGI_{120}	0.43 ***	0.363 **	0.205	0.162
IGI_{30}	0.606 ***	0.528 ***	0.495 ***	0.445 ***
IGI_{60}	0.544 ***	0.445 ***	0.271 **	0.266 *
I_{120}/I_0	-0.068	-0.057	-0.097	-0.075
I_{30}/I_0	0.248 *	0.284 **	0.164	0.188
I_{60}/I_0	0.111	0.125	0.096	0.125
Kadowaki model	0.629 ***	0.548 ***	0.501 ***	0.433 ***
$\log(I_0)$	0.431 ***	0.298 **	0.436 ***	0.333 **
$\log(I_{120})$	0.287 **	0.206	0.207 *	0.153
$\log(I_{30})$	0.63 ***	0.535 ***	0.56 ***	0.493 ***
$\log(I_{60})$	0.479 ***	0.371 ***	0.435 ***	0.368 ***
$\log(I_{90})$	0.307 **	0.184	0.238 *	0.153

CPI_0	0.356 ***	0.293 **	0.367 ***	0.319 **
CPI_{120}	0.452 ***	0.423 ***	0.312 **	0.326 **
first-phase Stumvoll	0.591 ***	0.498 ***	0.495 ***	0.406 ***
second-phase Stumvoll	0.608 ***	0.497 ***	0.494 ***	0.392 ***

In the IFG and/or IGT-group, the strongest correlation between Insulin $iAUC_{0\text{-}10}$ and C-peptide $iAUC_{0\text{-}10}$ was identified for AUC ($CP_{0\text{-}60}$)/AUC ($G_{0\text{-}60}$), CP_{30}, CP_{60}/G_{60} and CP_{30}/G_{30}.

Figure 2: OGTT-indices with the highest Spearman's and Pearson's correlation coefficients in subset with prediabetes compared to first-phase IVGTT

According to the analysis, CP_0 and AUC (I_{all}) have the weakest correlation with IVGTT-derived C-peptide $iAUC_{0\text{-}10}$ in a prediabetic group (Table 6). The lowest

significant correlation with IVGTT-derived insulin $iAUC_{0-10}$ was determined for CP_{120}, I_{120} and $\log(I_{120})$ (Table 6).

The correlation coefficients in a group of participants with test-diagnosed type 2 diabetes (n=16) were also analysed.

Table 7: Diabetes-group (n=16). Correlation between fasting and OGTT-derived insulin secretion indices and first-phase insulin secretion measured with the IVGTT (* p<0.05; **p<0.01; ***p<0.001).

Indices	Insulin $iAUC_{0-10}$ Spearman	C-peptide $iAUC_{0-10}$ Spearman	Insulin $iAUC_{0-10}$ Pearson	C-peptide $iAUC_{0-10}$ Pearson
AUC (CP_{0-120})/AUC (G_{0-120})	0.296	0.136	0.22	0.222
AUC(CP_{0-30})/AUC(G_{0-30})	0.165	0.033	0.049	0.063
AUC (CP_{0-60})/AUC (G_{0-60})	0.236	0.046	0.172	0.144
AUC(CP_{all})	0.186	0.021	0.071	0.035
AUC(I_{all})	0.243	0.125	0.209	0.227
AUC(I_{0-120})/AUC(G_{0-120})	0.443	0.321	0.339	0.373
AUC(I_{0-30})/AUC(G_{0-30})	0.468	0.368	0.409	0.346
AUC(I_{0-60})/AUC(G_{0-60})	0.35	0.204	0.213	0.243
BIGTT-$AIR_{0-30-120}$	0.382	0.593 *	0.411	0.525 *
BIGTT-$AIR_{0-60-120}$	0.175	0.396	0.173	0.306
CIR_{120}	0.582 *	0.521 *	0.293	0.381
CIR_{30}	0.668 **	0.579 *	0.607 *	0.573 *
CIR_{60}	0.493	0.354	0.33	0.346
CP_{120}/CP_0	0.196	0.218	0.436	0.411
CP_{30}/CP_0	0.684 **	0.556 *	0.807 ***	0.642 *
CP_{60}/CP_0	0.529 *	0.264	0.716 **	0.49
CP_0	-0.129	-0.182	-0.251	-0.214
CP_0/G_0	-0.007	-0.054	-0.12	-0.018

CP_{120}	0.029	-0.032	0.044	0.059
CP_{120}/G_{120}	0.214	0.204	0.194	0.26
CP_{30}	0.059	-0.147	0.063	-0.039
CP_{30}/G_{30}	0.314	0.138	0.189	0.146
CP_{60}	0.236	NA	0.103	0.009
CP_{60}/G_{60}	0.357	0.132	0.258	0.185
HOMA-%B	-0.139	0.029	-0.193	0.088
HOMA2-%B(CP)	0.107	0.186	0.057	0.228
HOMA2-%B(I)	-0.077	0.218	-0.144	0.211
I_0	-0.368	-0.286	-0.47	-0.319
I_0/G_0	-0.307	-0.154	-0.372	-0.151
I_{120}	0.075	0.036	0.191	0.226
I_{120}/G_{120}	0.325	0.282	0.287	0.345
I_{30}	0.404	0.239	0.358	0.208
ΔI_{30}	0.493	0.279	0.464	0.279
I_{30}/G_{30}	0.546 *	0.357	0.511	0.399
I_{60}	0.268	0.107	0.148	0.132
ΔI_{60}	0.332	0.132	0.209	0.173
I_{60}/G_{60}	0.389	0.196	0.266	0.267
IGI_{120}	0.196	0.174	0.164	0.206
IGI_{30}	0.682 **	0.432	0.608 *	0.428
IGI_{60}	0.454	0.196	0.296	0.252
I_{120}/I_0	0.379	0.336	0.601 *	0.521 *
I_{30}/I_0	0.611 *	0.382	0.642 **	0.376
I_{60}/I_0	0.843 ***	0.425	0.662 **	0.381
Kadowaki model	0.689 **	0.489	0.609 *	0.453
log(I_0)	-0.368	-0.286	-0.424	-0.252
log(I_{120})	0.075	0.036	0.133	0.187
log(I_{30})	0.404	0.239	0.357	0.219
log(I_{60})	0.268	0.107	0.13	0.13
log(I_{90})	0.264	0.204	0.091	0.222
CPI_0	-0.007	-0.054	-0.12	-0.018

CPI_{120}	0.214	0.204	0.194	0.26
first-phase Stumvoll	0.461	0.511	0.43	0.473
second-phase Stumvoll	0.179	0.082	0.056	-0.028

CP_{30}/CP_0, CP_{60}/CP_0, CIR_{30}, I_{30}/I_0, and BIGTT-$AIR_{0\text{-}30\text{-}120}$ correlated significantly with both Insulin $iAUC_{0\text{-}10}$ and C-peptide $iAUC_{0\text{-}10}$ (Figure 3, Table 7).

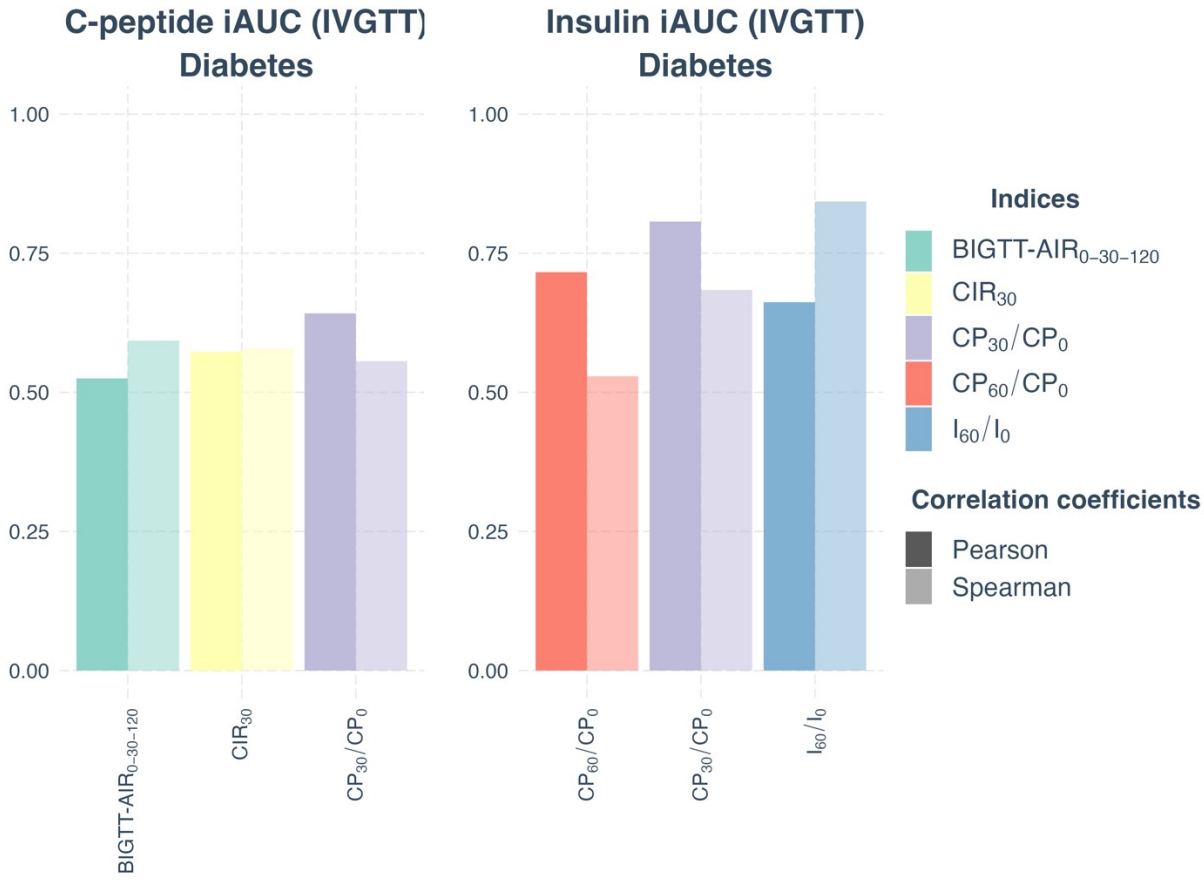


Figure 3: Top OGTT-indices by Spearman's and Pearson's correlation coefficients in subset with diabetes compared to first-phase IVGTT

Analysing the whole cohort of 316 subjects, without a classification into different glucose tolerance stages, I found multiple indices with a correlation coefficient >0.5 according to Spearman's and Pearson's tests (Figure 4).

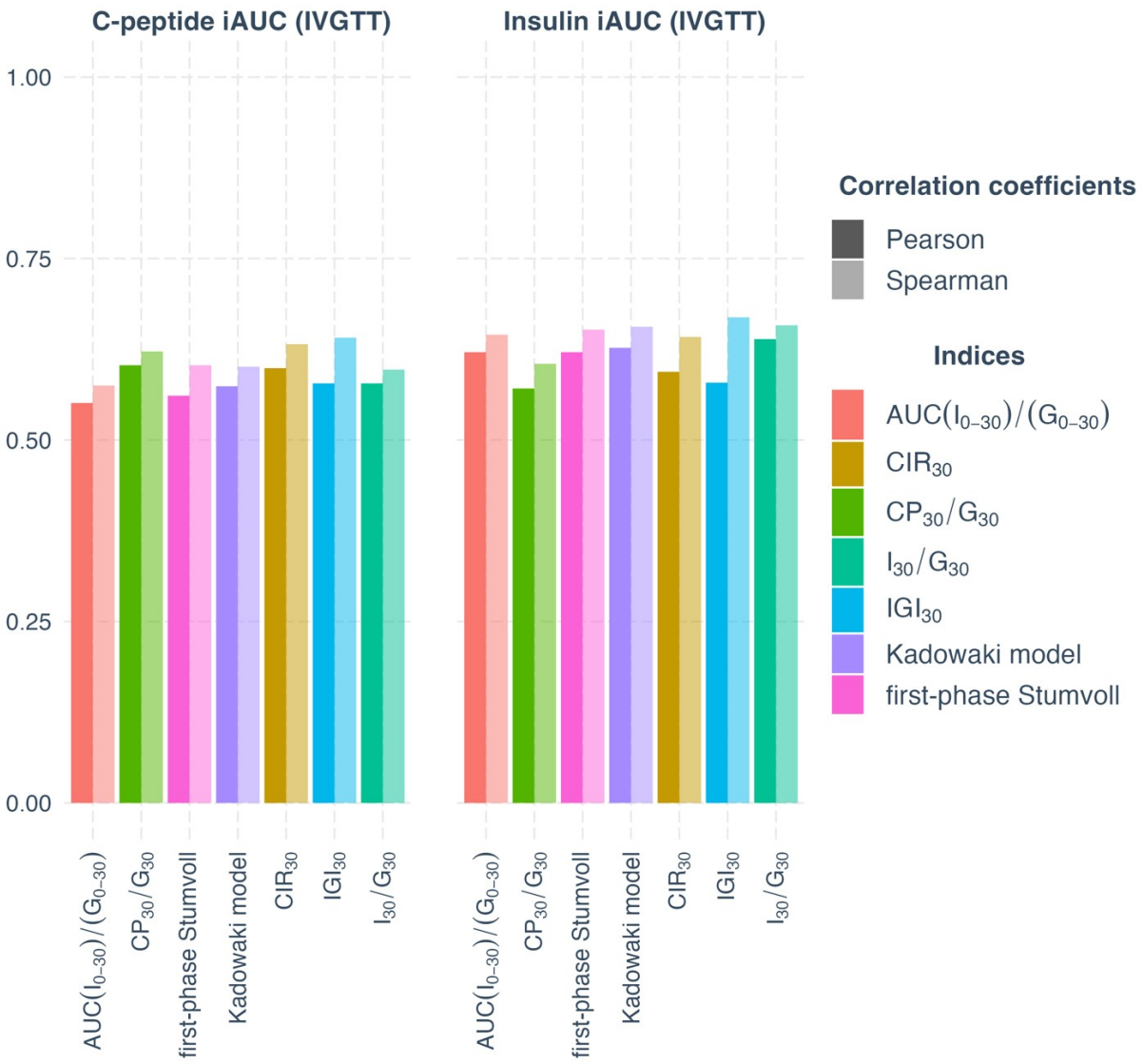


Figure 4: Top indices by Spearman's and Pearson's correlation coefficients in the entire cohort (n=316) compared to first-phase IVGTT

The highest correlation in the entire cohort was detected for AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), first-phase Stumvoll, Kadowaki model, IGI_{30}, CIR_{30} and I_{30}/G_{30} (Figure 4). The weakest correlation for both groups had IGI_{120} and CIR_{120}.

3.3 Comparison of insulin secretion indices with dynamic insulin secretion measurements in the hyperglycaemic clamp

I examined different formulas for computing insulin secretion from OGTT and hyperglycaemic clamp techniques in a group of 76 volunteers.
In a group of participants with normal glucose tolerance, most surrogate indices positively correlate with the first-phase insulin response obtained from the gold standard (Table 8).

Table 8: NGT-group (n=49). Correlation between fasting and OGTT-derived insulin secretion indices and first-phase insulin secretion measured with the hyperglycaemic clamp (* p<0.05; **p<0.01; ***p<0.001).

Indices	Insulin 1st-phase Spearman	C-peptide 1st-phase Spearman	Insulin 1st-phase Pearson	C-peptide 1st-phase Pearson
AUC (CP_{0-120})/AUC (G_{0-120})	0.508 ***	0.542 ***	0.694 ***	0.566 ***
AUC(CP_{0-30})/AUC(G_{0-30})	0.49 ***	0.524 ***	0.733 ***	0.655 ***
AUC (CP_{0-60})/AUC (G_{0-60})	0.579 ***	0.612 ***	0.711 ***	0.611 ***
AUC(CP_{all})	0.45 **	0.519 ***	0.63 ***	0.566 ***
AUC(I_{all})	0.593 ***	0.52 ***	0.728 ***	0.788 ***
AUC(I_{0-120})/AUC(G_{0-120})	0.65 ***	0.577 ***	0.857 ***	0.852 ***
AUC(I_{0-30})/AUC(G_{0-30})	0.764 ***	0.666 ***	0.913 ***	0.896 ***
AUC(I_{0-60})/AUC(G_{0-60})	0.596 ***	0.509 ***	0.665 ***	0.733 ***
BIGTT-$AIR_{0-30-120}$	0.692 ***	0.621 ***	0.919 ***	0.868 ***
BIGTT-$AIR_{0-60-120}$	0.576 ***	0.472 ***	0.718 ***	0.711 ***
CIR_{120}	0.526 ***	0.382 *	0.638 ***	0.508 ***
CIR_{30}	0.615 ***	0.555 ***	0.695 ***	0.63 ***
CIR_{60}	0.45 **	0.353 *	0.387 **	0.229
CP_{120}/CP_0	-0.184	-0.215	-0.192	-0.307 *
CP_{30}/CP_0	-0.044	-0.009	0.076	0.011
CP_{60}/CP_0	-0.037	-0.009	-0.063	-0.162

CP_0	0.412 **	0.457 **	0.415 **	0.58 ***
CP_0/G_0	0.513 ***	0.535 ***	0.661 ***	0.681 ***
CP_{120}	0.215	0.219	0.449 **	0.31 *
CP_{120}/G_{120}	0.46 **	0.517 ***	0.612 ***	0.499 ***
CP_{30}	0.411 **	0.465 ***	0.672 ***	0.661 ***
CP_{30}/G_{30}	0.43 **	0.462 ***	0.654 ***	0.546 ***
CP_{60}	0.437 **	0.524 ***	0.579 ***	0.533 ***
CP_{60}/G_{60}	0.492 ***	0.524 ***	0.638 ***	0.505 ***
HOMA-%B	0.577 ***	0.407 **	0.616 ***	0.686 ***
HOMA2-%B(CP)	0.517 ***	0.51 ***	0.658 ***	0.656 ***
HOMA2-%B(I)	0.49 **	0.494 **	0.668 ***	0.751 ***
I_0	0.473 ***	0.338 *	0.39 **	0.568 ***
I_0/G_0	0.498 ***	0.349 *	0.475 ***	0.632 ***
I_{120}	0.341 *	0.286 *	0.645 ***	0.53 ***
I_{120}/G_{120}	0.475 ***	0.397 **	0.755 ***	0.65 ***
I_{30}	0.706 ***	0.641 ***	0.902 ***	0.871 ***
ΔI_{30}	0.694 ***	0.626 ***	0.882 ***	0.85 ***
I_{30}/G_{30}	0.742 ***	0.663 ***	0.901 ***	0.885 ***
I_{60}	0.507 ***	0.454 **	0.496 ***	0.629 ***
ΔI_{60}	0.49 ***	0.454 **	0.48 ***	0.614 ***
I_{60}/G_{60}	0.612 ***	0.542 ***	0.722 ***	0.755 ***
IGI_{120}	0.538 ***	0.532 **	0.534 ***	0.483 **
IGI_{30}	0.632 ***	0.584 ***	0.678 ***	0.641 ***
IGI_{60}	0.42 **	0.401 **	0.234	0.255
I_{120}/I_0	-0.051	-0.021	0.15	0.015
I_{30}/I_0	0.134	0.263	0.364 *	0.41 **
I_{60}/I_0	0.115	0.181	0.077	0.1
Kadowaki model	0.702 ***	0.627 ***	0.875 ***	0.861 ***
$\log(I_0)$	0.504 ***	0.377 **	0.481 ***	0.534 ***
$\log(I_{120})$	0.341 *	0.286 *	0.466 ***	0.4 **
$\log(I_{30})$	0.706 ***	0.641 ***	0.794 ***	0.804 ***
$\log(I_{60})$	0.507 ***	0.454 **	0.456 **	0.538 ***

log(I_{90})	0.35 *	0.317 *	0.464 ***	0.46 ***
CPI_0	0.513 ***	0.535 ***	0.661 ***	0.681 ***
CPI_{120}	0.46 **	0.517 ***	0.612 ***	0.499 ***
first-phase Stumvoll	0.701 ***	0.636 ***	0.859 ***	0.867 ***
second-phase Stumvoll	0.658 ***	0.576 ***	0.864 ***	0.87 ***

In NGT-group, the highest significant association with first-phase insulin secretion measured by insulin and C-peptide was defined for AUC (I_{0-30})/AUC (G_{0-30}), I_{30}, I_{30}/G_{30} and BIGTT-$AIR_{0-30-120}$ (Table 8).

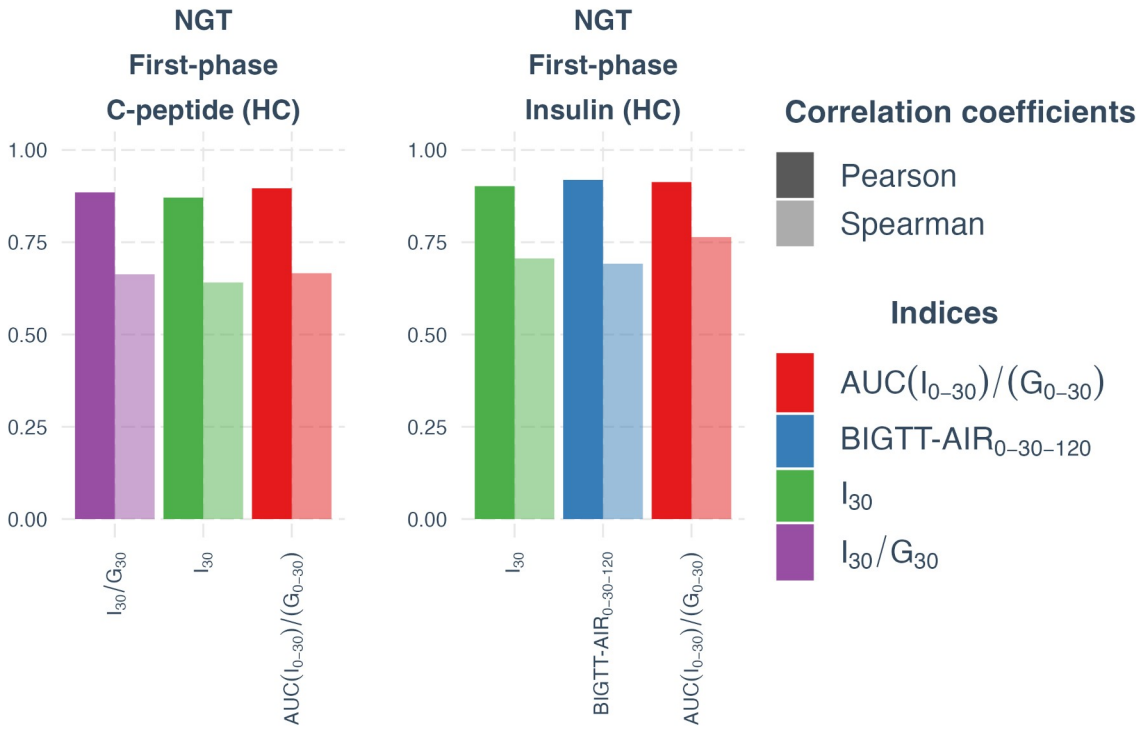


Figure 5: Indices with the highest Spearman's and Pearson's correlation coefficients in NGT group compared to first- phase hyperglycaemic clamp

In contrast, log(I_{90}) and log(I_{120}) have the lowest significant correlation coefficients in this group (Table 8).

The same analysis was done for IFG/IGT group. As well as in NGT subgroup, the majority of fasting- and OGTT-derived indices have strong correlation coefficients according to both Pearson's and Spearman's tests.

Table 9: IFG and/or IGT -group (n=23). Correlation between fasting and OGTT-derived insulin secretion indices and first-phase insulin secretion measured with the hyperglycaemic clamp (* p<0.05; **p<0.01; ***p<0.001).

Indices	Insulin 1st-phase Spearman	C-peptide 1st-phase Spearman	Insulin 1st-phase Pearson	C-peptide 1st-phase Pearson
AUC (CP_{0-120})/AUC (G_{0-120})	0.605 **	0.659 ***	0.589 **	0.655 ***
AUC(CP_{0-30})/AUC(G_{0-30})	0.768 ***	0.757 ***	0.788 ***	0.833 ***
AUC (CP_{0-60})/AUC (G_{0-60})	0.63 **	0.633 **	0.696 ***	0.729 ***
AUC(CP_{all})	0.657 **	0.646 **	0.477 *	0.575 **
AUC(I_{all})	0.582 **	0.517 *	0.51 *	0.611 **
AUC(I_{0-120})/AUC(G_{0-120})	0.657 ***	0.596 **	0.619 **	0.704 ***
AUC(I_{0-30})/AUC(G_{0-30})	0.704 ***	0.639 **	0.825 ***	0.852 ***
AUC(I_{0-60})/AUC(G_{0-60})	0.649 **	0.583 **	0.67 ***	0.735 ***
BIGTT-$AIR_{0-30-120}$	0.634 **	0.626 **	0.851 ***	0.868 ***
BIGTT-$AIR_{0-60-120}$	0.78 ***	0.745 ***	0.8 ***	0.828 ***
CIR_{120}	0.601 **	0.633 **	0.33	0.42
CIR_{30}	0.67 ***	0.653 ***	0.725 ***	0.71 ***
CIR_{60}	0.575 **	0.523 *	-0.061	-0.095
CP_{120}/CP_0	-0.036	0.106	-0.236	-0.112
CP_{30}/CP_0	0.234	0.212	0.017	0.111
CP_{60}/CP_0	0.091	0.051	-0.119	-0.061
CP_0	0.541 **	0.524 *	0.731 ***	0.749 ***
CP_0/G_0	0.649 **	0.628 **	0.723 ***	0.76 ***
CP_{120}	0.452 *	0.57 **	0.229	0.339
CP_{120}/G_{120}	0.401	0.508 *	0.129	0.2
CP_{30}	0.764 ***	0.718 ***	0.722 ***	0.785 ***
CP_{30}/G_{30}	0.742 ***	0.742 ***	0.751 ***	0.789 ***
CP_{60}	0.613 **	0.581 **	0.541 **	0.604 **
CP_{60}/G_{60}	0.504 *	0.508 *	0.559 **	0.565 **
HOMA-%B	0.688 ***	0.608 **	0.733 ***	0.784 ***
HOMA2-%B(CP)	0.704 ***	0.718 ***	0.59 **	0.665 ***

HOMA2-%B(I)	0.678 ***	0.574 **	0.706 ***	0.76 ***
I_0	0.618 **	0.511 *	0.769 ***	0.8 ***
I_0/G_0	0.695 ***	0.607 **	0.771 ***	0.808 ***
I_{120}	0.633 **	0.636 **	0.399	0.529 **
I_{120}/G_{120}	0.623 **	0.615 **	0.437 *	0.567 **
I_{30}	0.669 ***	0.6 **	0.775 ***	0.813 ***
ΔI_{30}	0.63 **	0.593 **	0.753 ***	0.79 ***
I_{30}/G_{30}	0.676 ***	0.648 ***	0.83 ***	0.845 ***
I_{60}	0.561 **	0.503 *	0.555 **	0.637 **
ΔI_{60}	0.537 **	0.477 *	0.522 *	0.607 **
I_{60}/G_{60}	0.645 **	0.577 **	0.698 ***	0.753 ***
IGI_{120}	0.219	0.256	0.156	0.229
IGI_{30}	0.625 **	0.615 **	0.461 *	0.437 *
IGI_{60}	0.723 ***	0.641 ***	0.426 *	0.453 *
I_{120}/I_0	0.146	0.205	-0.08	0.031
I_{30}/I_0	0.268	0.268	0.064	0.089
I_{60}/I_0	0.166	0.141	-0.007	0.057
Kadowaki model	0.636 **	0.608 **	0.803 ***	0.817 ***
$\log(I_0)$	0.618 **	0.511 *	0.705 ***	0.746 ***
$\log(I_{120})$	0.633 **	0.636 **	0.471 *	0.586 **
$\log(I_{30})$	0.669 ***	0.6 **	0.651 ***	0.705 ***
$\log(I_{60})$	0.561 **	0.503 *	0.47 *	0.509 *
$\log(I_{90})$	0.404	0.357	0.289	0.381
CPI_0	0.649 **	0.628 **	0.723 ***	0.76 ***
CPI_{120}	0.401	0.508 *	0.129	0.2
first-phase Stumvoll	0.644 **	0.617 **	0.796 ***	0.818 ***
second-phase Stumvoll	0.615 **	0.559 **	0.772 ***	0.809 ***

The strongest positive relationship between first-phase clamp- and OGTT-derived values in prediabetes belong to AUC (I_{0-30})/AUC (G_{0-30}), I_{30}/G_{30}, BIGTT-$AIR_{0-30-120}$, Kadowaki model (Figure 3, Table 9).

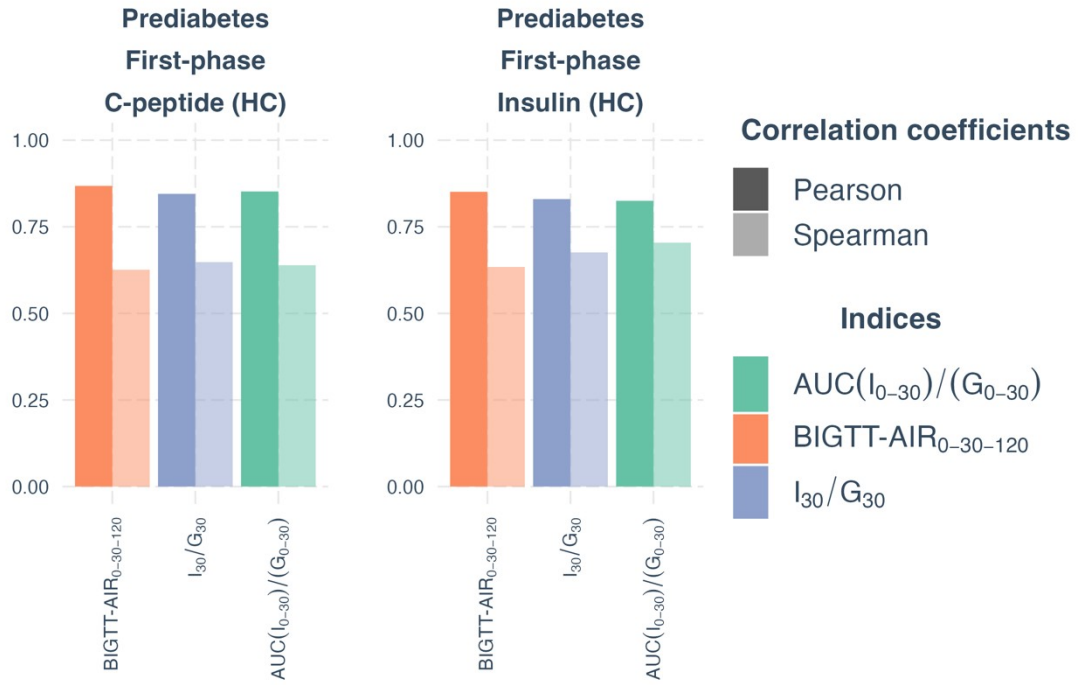


Figure 6: Indices with the highest Spearman's and Pearson's correlation coefficients in IFG and/or IGT group compared to first-phase hyperglycaemic clamp (HC).

The lowest significant correlation was determined for log(I_{60}) and IGI_{30} in this group (Table 9).

In agreement with the obtaining data the second-phase C-peptide and insulin highly correlate with most of surrogate indices in non-impaired glucose tolerance group.

Table 10: NGT-group (n=49). Correlation between fasting and OGTT-derived insulin secretion indices and second-phase insulin secretion measured with the hyperglycaemic clamp (* p<0.05; **p<0.01; ***p<0.001).

Indices	Insulin 2^{d}-phase Spearman	C-peptide 2^{d}-phase Spearman	Insulin 2^{d}-phase Pearson	C-peptide 2^{d}-phase Pearson
AUC(CP_{0-120})/AUC(G_{0-120})	0.585 ***	0.578 ***	0.505 ***	0.59 ***
AUC(CP_{0-30})/AUC(G_{0-30})	0.518 ***	0.5 ***	0.557 ***	0.624 ***
AUC(CP_{0-60})/AUC(G_{0-60})	0.593 ***	0.61 ***	0.564 ***	0.619 ***
AUC(CP_{all})	0.648 ***	0.664 ***	0.566 ***	0.643 ***
AUC(I_{all})	0.746 ***	0.605 ***	0.837 ***	0.751 ***
AUC(I_{0-120})/AUC(G_{0-120})	0.785 ***	0.646 ***	0.887 ***	0.809 ***
AUC(I_{0-30})/AUC(G_{0-30})	0.748 ***	0.593 ***	0.902 ***	0.766 ***
AUC(I_{0-60})/AUC(G_{0-60})	0.711 ***	0.579 ***	0.779 ***	0.678 ***
BIGTT-$AIR_{0-30-120}$	0.586 ***	0.43 **	0.855 ***	0.81 ***
BIGTT-$AIR_{0-60-120}$	0.53 ***	0.357 *	0.701 ***	0.663 ***
CIR_{120}	0.531 ***	0.445 **	0.573 ***	0.502 ***
CIR_{30}	0.626 ***	0.51 ***	0.542 ***	0.566 ***
CIR_{60}	0.523 ***	0.398 **	0.397 **	0.366 *
CP_{120}/CP_0	-0.061	-0.2	-0.244	-0.279
CP_{30}/CP_0	-0.03	-0.08	-0.055	-0.108
CP_{60}/CP_0	0.014	-0.009	-0.104	-0.143
CP_0	0.454 **	0.516 ***	0.537 ***	0.572 ***
CP_0/G_0	0.466 ***	0.525 ***	0.657 ***	0.703 ***
CP_{120}	0.419 **	0.352 *	0.355 *	0.44 **
CP_{120}/G_{120}	0.498 ***	0.57 ***	0.477 ***	0.578 ***
CP_{30}	0.503 ***	0.509 ***	0.592 ***	0.587 ***
CP_{30}/G_{30}	0.463 **	0.43 **	0.431 **	0.517 ***
CP_{60}	0.605 ***	0.636 ***	0.554 ***	0.608 ***
CP_{60}/G_{60}	0.539 ***	0.546 ***	0.428 **	0.508 ***
HOMA-%B	0.48 ***	0.318 *	0.692 ***	0.563 ***
HOMA2-%B(CP)	0.403 **	0.42 **	0.6 ***	0.581 ***

HOMA2-%B(I)	0.429 **	0.401 *	0.727 ***	0.709 ***
I_0	0.542 ***	0.391 **	0.586 ***	0.47 ***
I_0/G_0	0.539 ***	0.38 **	0.645 ***	0.499 ***
I_{120}	0.585 ***	0.37 **	0.611 ***	0.631 ***
I_{120}/G_{120}	0.686 ***	0.518 ***	0.721 ***	0.733 ***
I_{30}	0.704 ***	0.595 ***	0.901 ***	0.718 ***
ΔI_{30}	0.684 ***	0.571 ***	0.881 ***	0.689 ***
I_{30}/G_{30}	0.732 ***	0.587 ***	0.882 ***	0.77 ***
I_{60}	0.656 ***	0.543 ***	0.683 ***	0.594 ***
ΔI_{60}	0.651 ***	0.545 ***	0.671 ***	0.577 ***
I_{60}/G_{60}	0.725 ***	0.606 ***	0.8 ***	0.714 ***
IGI_{120}	0.578 ***	0.564 ***	0.534 ***	0.458 **
IGI_{30}	0.683 ***	0.569 ***	0.583 ***	0.595 ***
IGI_{60}	0.617 ***	0.546 ***	0.332 *	0.356 *
I_{120}/I_0	0.137	0.058	0.085	0.104
I_{30}/I_0	0.146	0.189	0.335 *	0.304 *
I_{60}/I_0	0.263	0.264	0.162	0.133
Kadowaki model	0.696 ***	0.542 ***	0.858 ***	0.737 ***
$\log(I_0)$	0.569 ***	0.427 **	0.571 ***	0.524 ***
$\log(I_{120})$	0.585 ***	0.37 **	0.515 ***	0.488 ***
$\log(I_{30})$	0.704 ***	0.595 ***	0.809 ***	0.705 ***
$\log(I_{60})$	0.656 ***	0.543 ***	0.604 ***	0.553 ***
$\log(I_{90})$	0.564 ***	0.468 ***	0.539 ***	0.557 ***
CPI_0	0.466 ***	0.525 ***	0.657 ***	0.703 ***
CPI_{120}	0.498 ***	0.57 ***	0.477 ***	0.578 ***
first-phase Stumvoll	0.717 ***	0.577 ***	0.87 ***	0.761 ***
second-phase Stumvoll	0.675 ***	0.581 ***	0.905 ***	0.732 ***

The strongest relationship between second-phase clamp- and OGTT-derived measurements in NGT group correspond to AUC (I_{0-30})/AUC (G_{0-30}), AUC (I_{0-120})/AUC (G_{0-120}), I_{30}, I_{30}/G_{30}, BIGTT-$AIR_{0-30-120}$ (Figure 7, Table 10).

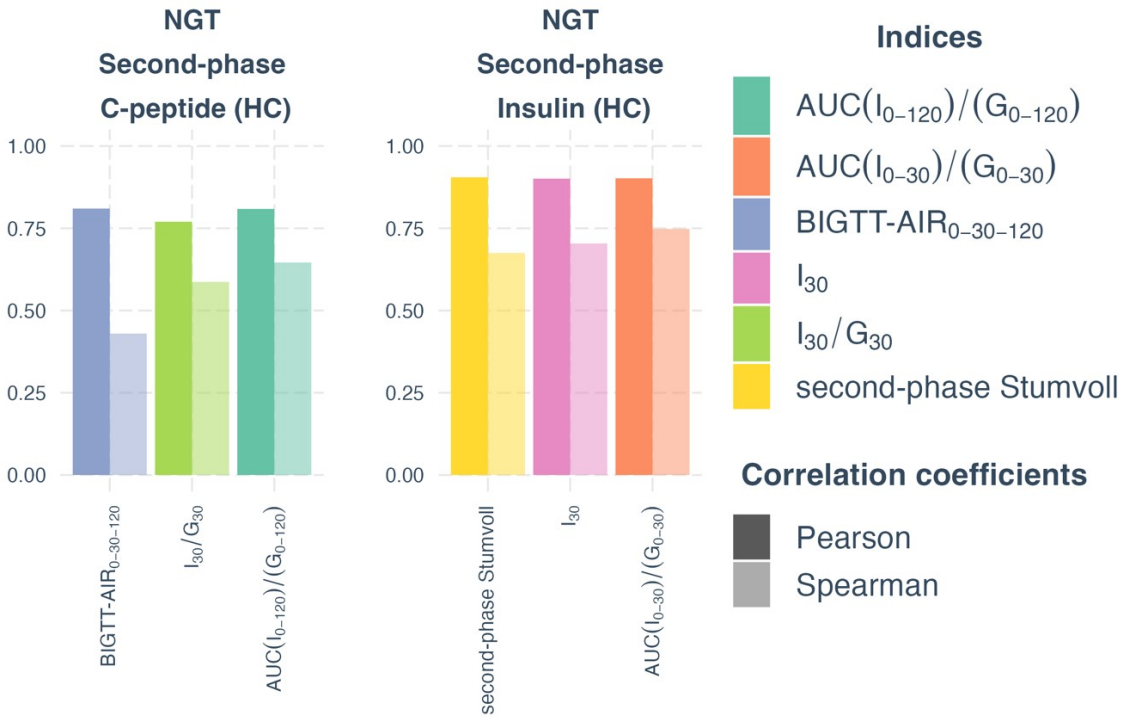


Figure 7: Indices with the highest Spearman's and Pearson's correlation coefficients in NGT group compared to second-phase hyperglycaemic clamp.

For the group with impaired glucose metabolism, the strong association with second-phase was determined for the majority of measures. The entire set of correlation indices for the IFG/IGT group was presented in a table below.

Table 11: IFG and/or IGT -group (n=23). Correlation between fasting and OGTT-derived insulin secretion indices and second-phase insulin secretion measured with the hyperglycaemic clamp (* p<0.05; **p<0.01; ***p<0.001).

Indices	Insulin 2^{nd}-phase Spearman	C-peptide 2^{nd}-phase Spearman	Insulin 2^{nd}-phase Pearson	C-peptide 2^{nd}-phase Pearson
$AUC(CP_{0-120})/AUC(G_{0-120})$	0.457 *	0.527 *	0.61 **	0.618 **
$AUC(CP_{0-30})/AUC(G_{0-30})$	0.64 **	0.617 **	0.802 ***	0.756 ***
$AUC(CP_{0-60})/AUC(G_{0-60})$	0.502 *	0.486 *	0.695 ***	0.646 **
$AUC(CP_{all})$	0.527 *	0.45 *	0.505 *	0.579 **
$AUC(I_{all})$	0.549 **	0.397	0.599 **	0.693 ***
$AUC(I_{0-120})/AUC(G_{0-120})$	0.598 **	0.511 *	0.699 ***	0.755 ***
$AUC(I_{0-30})/AUC(G_{0-30})$	0.624 **	0.611 **	0.855 ***	0.838 ***
$AUC(I_{0-60})/AUC(G_{0-60})$	0.628 **	0.514 *	0.725 ***	0.757 ***
$BIGTT\text{-}AIR_{0-30-120}$	0.617 **	0.632 **	0.874 ***	0.853 ***
$BIGTT\text{-}AIR_{0-60-120}$	0.736 ***	0.73 ***	0.823 ***	0.807 ***
CIR_{120}	0.581 **	0.578 **	0.462 *	0.479 *
CIR_{30}	0.608 **	0.651 **	0.739 ***	0.663 ***
CIR_{60}	0.565 **	0.533 **	-0.067	-0.085
CP_{120}/CP_0	-0.135	0.099	-0.27	0.016
CP_{30}/CP_0	0.155	0.209	-0.037	0.171
CP_{60}/CP_0	0.09	0.046	-0.154	0.003
CP_0	0.433 *	0.324	0.775 ***	0.667 ***
CP_0/G_0	0.484 *	0.411	0.742 ***	0.67 ***
CP_{120}	0.249	0.405	0.234	0.401
CP_{120}/G_{120}	0.25	0.412	0.158	0.25
CP_{30}	0.631 **	0.535 *	0.726 ***	0.723 ***
CP_{30}/G_{30}	0.612 **	0.636 **	0.763 ***	0.721 ***
CP_{60}	0.51 *	0.411	0.543 **	0.568 **
CP_{60}/G_{60}	0.397	0.382	0.561 **	0.493 *
HOMA-%B	0.66 ***	0.552 **	0.766 ***	0.81 ***

HOMA2-%B(CP)	0.519 *	0.537 *	0.563 **	0.58 **
HOMA2-%B(I)	0.64 **	0.52 *	0.734 ***	0.787 ***
I_0	0.621 **	0.463 *	0.842 ***	0.832 ***
I_0/G_0	0.678 ***	0.548 **	0.831 ***	0.837 ***
I_{120}	0.541 **	0.509 *	0.466 *	0.643 ***
I_{120}/G_{120}	0.547 **	0.517 *	0.531 **	0.68 ***
I_{30}	0.58 **	0.545 **	0.807 ***	0.81 ***
ΔI_{30}	0.557 **	0.552 **	0.775 ***	0.781 ***
I_{30}/G_{30}	0.587 **	0.624 **	0.855 ***	0.818 ***
I_{60}	0.536 **	0.433 *	0.622 **	0.682 ***
ΔI_{60}	0.503 *	0.403	0.586 **	0.653 ***
I_{60}/G_{60}	0.593 **	0.509 *	0.753 ***	0.763 ***
IGI_{120}	0.273	0.319	0.233	0.371
IGI_{30}	0.534 **	0.602 **	0.466 *	0.407
IGI_{60}	0.733 ***	0.6 **	0.555 **	0.414 *
I_{120}/I_0	0.04	0.077	-0.111	0.066
I_{30}/I_0	0.213	0.28	0.037	0.081
I_{60}/I_0	0.132	0.093	-0.025	0.064
Kadowaki model	0.543 **	0.567 **	0.82 ***	0.785 ***
$\log(I_0)$	0.621 **	0.463 *	0.766 ***	0.759 ***
$\log(I_{120})$	0.541 **	0.509 *	0.497 *	0.612 **
$\log(I_{30})$	0.58 **	0.545 **	0.679 ***	0.701 ***
$\log(I_{60})$	0.536 **	0.433 *	0.505 *	0.499 *
$\log(I_{90})$	0.425 *	0.265	0.37	0.412
CPI_0	0.484 *	0.411	0.742 ***	0.67 ***
CPI_{120}	0.25	0.412	0.158	0.25
first-phase Stumvoll	0.58 **	0.574 **	0.846 ***	0.827 ***
second-phase Stumvoll	0.548 **	0.468 *	0.814 ***	0.812 ***

I_0/G_0, AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), BIGTT-AIR$_{0\text{-}30\text{-}120}$, I_{30}/G_{30}, first-phase Stumvoll have the highest correlation coefficients compared with the second-phase insulin secretion during hyperglycaemic clamp in prediabetes group (Figure 8, Table 11).

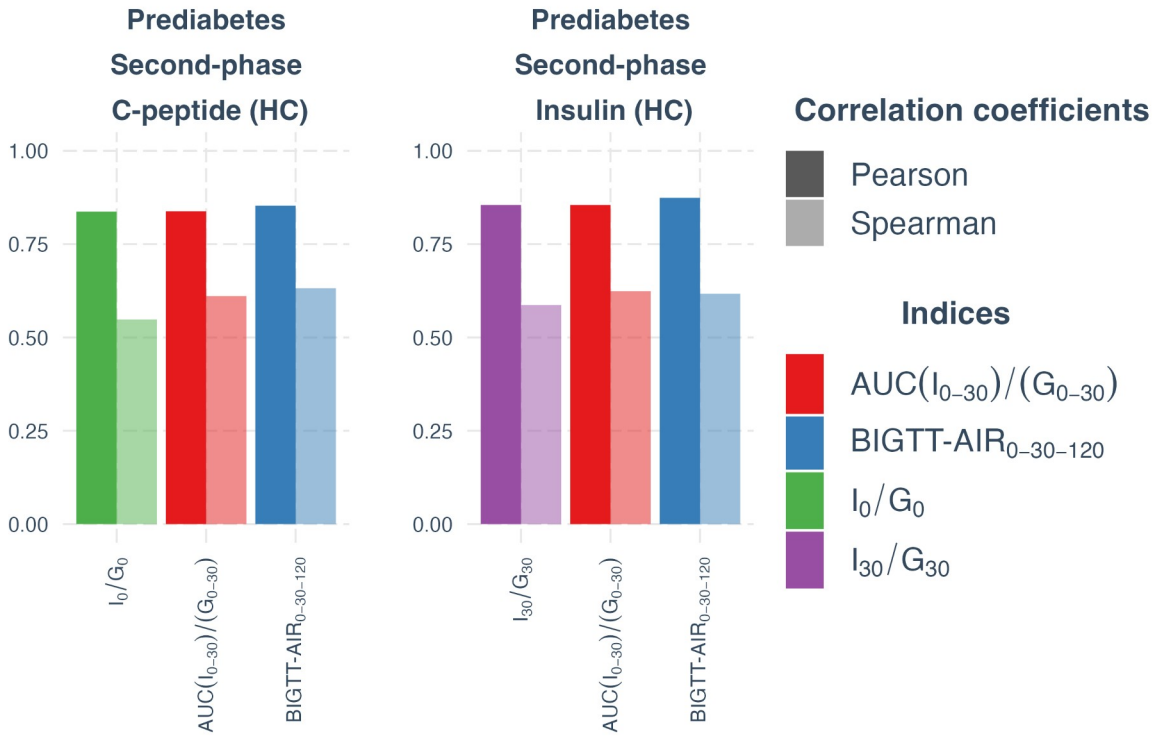


Figure 8: Indices with the highest Spearman's and Pearson's correlation coefficients in the IFG/IGT group compared to second- phase hyperglycaemic clamp

In the entire cohort comprising volunteers with and without impaired glucose metabolism, AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), BIGTT-AIR$_{0\text{-}30\text{-}120}$, I_{30}/G_{30}, I_{30} and first-phase Stumvoll showed the strongest correlation with first-phase and second-phase of insulin secretion according to both correlation tests (Figure 9). The surrogate measures with the lowest correlation were log(I_{120}), CP_{120} and log(I_{90}).

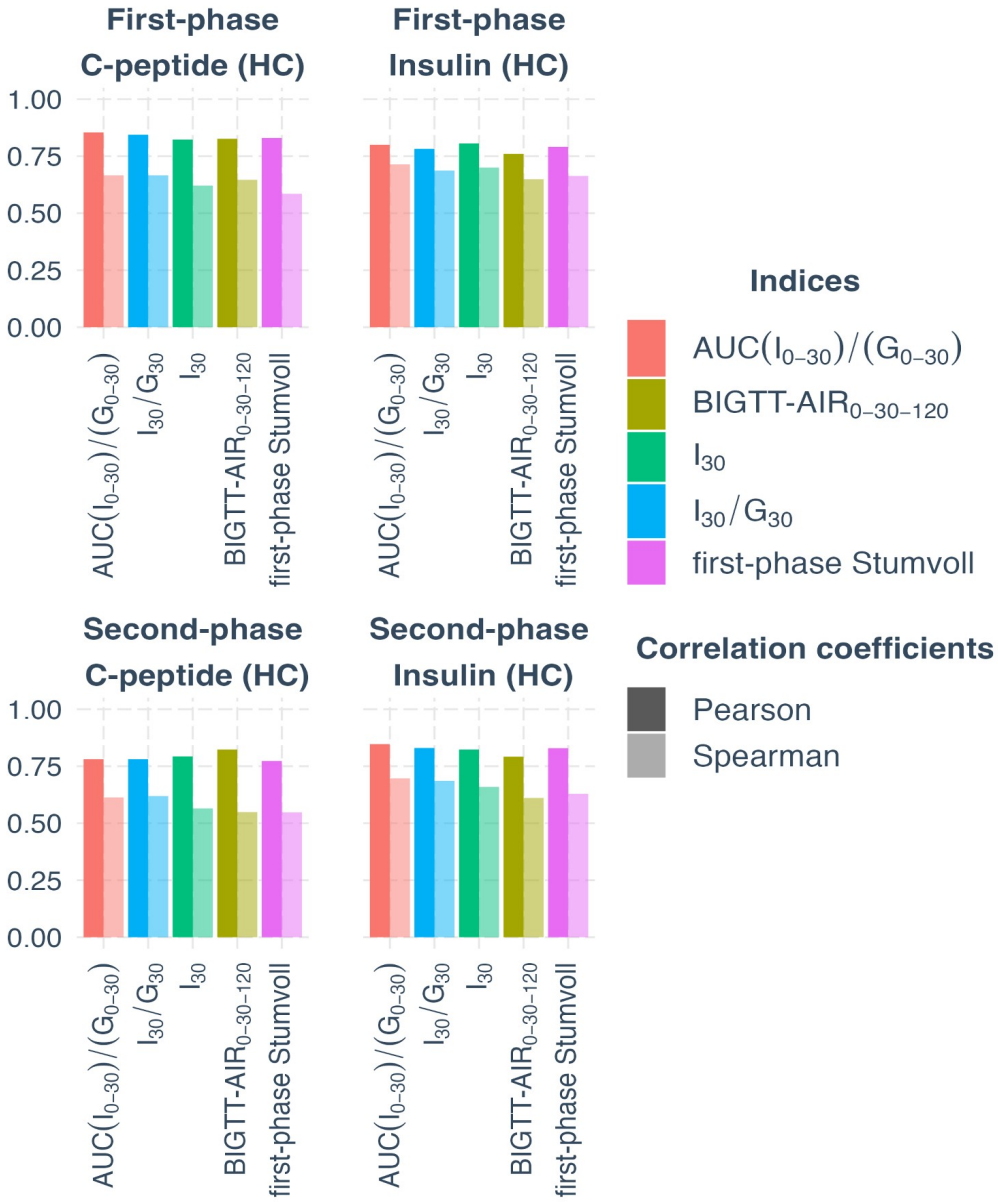


Figure 9: Indices with the highest Spearman's and Pearson's correlation coefficients in the whole group (n=76) compared to first- and second-phase hyperglycaemic clamp

The group of participants with newly diagnosed diabetes comprised only 4 subjects and was not included in the analysis.

The comparison of surrogate indices measuring insulin secretion using OGTT with the gold standard methods showed the highest Pearson's and Spearman's coefficients for I_{30}/G_{30}, AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), first-phase Stumvoll, Kadowaki

model in two phases and during all tests for both NGT and prediabetes groups (Figure 10).

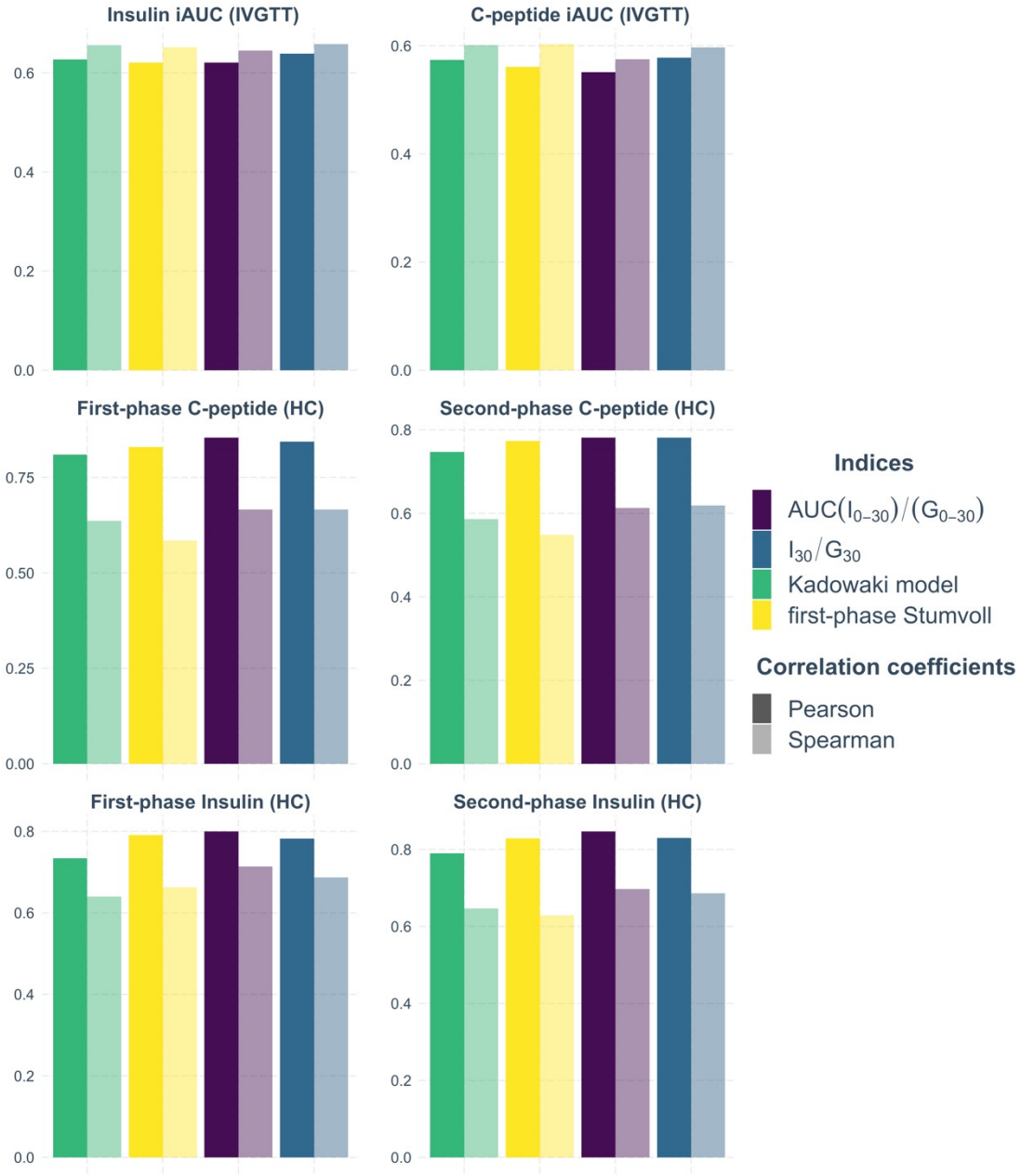


Figure 10: Indices with the highest Spearman's and Pearson's correlation coefficients in prediabetes and NGT groups in both study cohorts. Adapted from Katsiaryna Prystupa et al. 2022.

The CIR_{120} and IGI_{120} are the overall indices with the lowest significant correlation compared to both insulin and C-peptide based gold standard measurements according to both correlations' methods in both groups.

3.4 GLP-1-induced insulin (C-peptide) secretion

The modified hyperglycaemic clamp with the injection of GLP-1 agonist (PolyPeptide, WolfenbuÈttel, Germany) (Fritsche et al. 2000), also allowed us to correlate GLP-1 stimulated increase of insulin secretion both with C-peptide and insulin as readouts, with different OGTT-derived indices.

In the group of nonimpaired glucose tolerance, majority of indices correlate significantly with GLP-1-stimulated insulin release measured by insulin and C-peptide (Table 12).

Table 12: Correlation between fasting and OGTT-derived insulin secretion indices and GLP-1-stimulated insulin (C-peptide) secretion in NGT group (* p<0.05; **p<0.01; ***p<0.001).

Indices	Insulin GLP1 Spearman	C-peptide GLP1 Spearman	Insulin GLP1 Pearson	C-peptide GLP1 Pearson
AUC (CP_{0-120})/AUC (G_{0-120})	0.525 ***	0.38 **	0.511 ***	0.45 **
AUC(CP_{0-30})/ AUC(G_{0-30})	0.509 ***	0.395 **	0.607 ***	0.565 ***
AUC (CP_{0-60})/AUC (G_{0-60})	0.541 ***	0.392 **	0.546 ***	0.483 ***
AUC(CP_{all})	0.484 ***	0.343 *	0.526 ***	0.48 ***
AUC(I_{all})	0.613 ***	0.414 **	0.79 ***	0.638 ***
AUC(I_{0-120})/AUC(G_{0-120})	0.723 ***	0.529 ***	0.789 ***	0.61 ***
AUC(I_{0-30})/AUC(G_{0-	0.728 ***	0.54 ***	0.775 ***	0.57 ***

$_{30}$)				
AUC(I_{0-60})/AUC(G_{0-60})	0.649 ***	0.441 **	0.756 ***	0.59 ***
BIGTT-AIR$_{0-30-120}$	0.631 ***	0.466 ***	0.715 ***	0.6 ***
BIGTT-AIR$_{0-60-120}$	0.599 ***	0.376 **	0.761 ***	0.703 ***
CIR$_{120}$	0.435 **	0.32 *	0.378 *	0.266
CIR$_{30}$	0.669 ***	0.523 ***	0.545 ***	0.41 **
CIR$_{60}$	0.51 ***	0.335 *	0.123	-0.037
CP_{120}/CP_0	-0.252	-0.372 **	-0.352 *	-0.364 *
CP_{30}/CP_0	-0.031	-0.038	-0.077	-0.107
CP_{60}/CP_0	-0.05	-0.103	-0.161	-0.207
CP_0	0.361 *	0.304 *	0.496 ***	0.369 *
CP_0/G_0	0.398 **	0.357 *	0.678 ***	0.691 ***
CP_{120}	0.182	-0.023	0.32 *	0.321 *
CP_{120}/G_{120}	0.386 **	0.29 *	0.409 **	0.415 **
CP_{30}	0.445 **	0.355 *	0.568 ***	0.494 ***
CP_{30}/G_{30}	0.506 ***	0.397 **	0.507 ***	0.455 **
CP_{60}	0.512 ***	0.387 **	0.522 ***	0.453 **
CP_{60}/G_{60}	0.539 ***	0.38 **	0.445 **	0.371 **
HOMA-%B	0.431 **	0.304 *	0.674 ***	0.59 ***
HOMA2-%B(CP)	0.323 *	0.291 *	0.552 ***	0.531 ***
HOMA2-%B(I)	0.34 *	0.344 *	0.685 ***	0.668 ***
I_0	0.471 ***	0.29 *	0.598 ***	0.379 **
I_0/G_0	0.48 ***	0.304 *	0.623 ***	0.407 **
I_{120}	0.411 **	0.174	0.583 ***	0.542 ***
I_{120}/G_{120}	0.528 ***	0.314 *	0.64 ***	0.568 ***
I_{30}	0.663 ***	0.519 ***	0.734 ***	0.507 ***
ΔI_{30}	0.637 ***	0.498 ***	0.689 ***	0.449 **
I_{30}/G_{30}	0.747 ***	0.567 ***	0.757 ***	0.548 ***
I_{60}	0.556 ***	0.362 *	0.721 ***	0.579 ***
ΔI_{60}	0.55 ***	0.359 *	0.697 ***	0.544 ***
I_{60}/G_{60}	0.696 ***	0.498 ***	0.748 ***	0.561 ***

IGI_{120}	0.568 ***	0.541 ***	0.295	0.247
IGI_{30}	0.743 ***	0.578 ***	0.583 ***	0.413 **
IGI_{60}	0.524 ***	0.344 *	0.197	0.098
I_{120}/I_0	-0.017	-0.119	-0.078	-0.137
I_{30}/I_0	0.13	0.162	0.206	0.112
I_{60}/I_0	0.198	0.121	0.124	0.031
Kadowaki model	0.701 ***	0.527 ***	0.707 ***	0.486 ***
$\log(I_0)$	0.503 ***	0.333 *	0.644 ***	0.556 ***
$\log(I_{120})$	0.411 **	0.174	0.461 ***	0.319 *
$\log(I_{30})$	0.663 ***	0.519 ***	0.737 ***	0.56 ***
$\log(I_{60})$	0.556 ***	0.362 *	0.627 ***	0.489 ***
$\log(I_{90})$	0.319 *	0.185	0.454 **	0.384 **
CPI_0	0.398 **	0.357 *	0.678 ***	0.691 ***
CPI_{120}	0.386 **	0.29 *	0.409 **	0.415 **
first-phase Stumvoll	0.739 ***	0.573 ***	0.815 ***	0.617 ***
second-phase Stumvoll	0.6 ***	0.468 ***	0.776 ***	0.582 ***

The strongest correlation has I_{30}/G_{30}, followed by first-phase Stumvoll, AUC ($I_{0\text{-}120}$)/AUC ($G_{0\text{-}120}$), AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), and BIGTT-$AIR_{0\text{-}60\text{-}120}$ (Table 12).
The weakest correlation in NGT have CP_0, CP_{120}/G_{120} and CPI_{120}.
In the group with impaired glucose metabolism, most of the OGTT-derived indices have a significant linear and monotonic correlation with GLP-1-stimulated insulin secretion measurements.
The top-three surrogate indices with the strongest correlation in the prediabetes group are AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), first-phase Stumvoll, AUC ($I_{0\text{-}120}$)/AUC ($G_{0\text{-}120}$) (Table 13).

Table 13: Correlation between fasting and OGTT-derived insulin secretion indices and GLP-1-stimulated insulin (C-peptide) secretion in the IFG/IGT group (* p<0.05; **p<0.01; ***p<0.001).

Indices	Insulin GLP1 Spearman	C-peptide GLP1 Spearman	Insulin GLP1 Pearson	C-peptide GLP1 Pearson
AUC(CP_{0-120})/AUC(G_{0-120})	0.652 **	0.412	0.669 ***	0.523 *
AUC(CP_{0-30})/ AUC(G_{0-30})	0.736 ***	0.557 **	0.768 ***	0.624 **
AUC(CP_{0-60})/AUC(G_{0-60})	0.651 **	0.374	0.685 ***	0.541 **
AUC(CP_{all})	0.49 *	0.241	0.545 **	0.454 *
AUC(I_{all})	0.483 *	0.269	0.681 ***	0.573 **
AUC(I_{0-120})/ AUC(G_{0-120})	0.664 ***	0.414	0.75 ***	0.615 **
AUC(I_{0-30})/AUC(G_{0-30})	0.725 ***	0.517 *	0.799 ***	0.706 ***
AUC(I_{0-60})/AUC(G_{0-60})	0.588 **	0.327	0.717 ***	0.587 **
BIGTT-$AIR_{0-30-120}$	0.66 ***	0.49 *	0.771 ***	0.675 ***
BIGTT-$AIR_{0-60-120}$	0.682 ***	0.519 *	0.741 ***	0.619 **
CIR_{120}	0.608 **	0.38	0.529 *	0.318
CIR_{30}	0.777 ***	0.642 **	0.702 ***	0.61 **
CIR_{60}	0.758 ***	0.427 *	0.063	0.157
CP_{120}/CP_0	-0.119	0.033	-0.169	0.047
CP_{30}/CP_0	0.238	0.317	0.048	0.332
CP_{60}/CP_0	-0.005	0.015	-0.123	0.076
CP_0	0.489 *	0.19	0.691 ***	0.436 *
CP_0/G_0	0.516 *	0.269	0.677 ***	0.464 *
CP_{120}	0.292	0.234	0.312	0.303
CP_{120}/G_{120}	0.5 *	0.321	0.252	0.185

CP_{30}	0.606 **	0.456 *	0.685 ***	0.602 **
CP_{30}/G_{30}	0.765 ***	0.617 **	0.75 ***	0.63 **
CP_{60}	0.431 *	0.228	0.51 *	0.437 *
CP_{60}/G_{60}	0.595 **	0.334	0.608 **	0.489 *
HOMA-%B	0.565 **	0.349	0.755 ***	0.649 ***
HOMA2-%B(CP)	0.582 **	0.396	0.553 **	0.457 *
HOMA2-%B(I)	0.543 *	0.296	0.729 ***	0.634 **
I_0	0.544 **	0.262	0.809 ***	0.647 ***
I_0/G_0	0.582 **	0.329	0.805 ***	0.66 ***
I_{120}	0.339	0.358	0.584 **	0.561 **
I_{120}/G_{120}	0.529 *	0.416 *	0.656 ***	0.587 **
I_{30}	0.642 **	0.488 *	0.759 ***	0.689 ***
ΔI_{30}	0.609 **	0.487 *	0.726 ***	0.676 ***
I_{30}/G_{30}	0.726 ***	0.556 **	0.792 ***	0.698 ***
I_{60}	0.411	0.204	0.641 ***	0.527 **
ΔI_{60}	0.363	0.186	0.611 **	0.504 *
I_{60}/G_{60}	0.627 **	0.363	0.744 ***	0.6 **
IGI_{120}	0.358	0.155	0.374	0.316
IGI_{30}	0.718 ***	0.581 **	0.484 *	0.473 *
IGI_{60}	0.6 **	0.235	0.665 ***	0.184
I_{120}/I_0	-0.113	0.089	-0.073	0.04
I_{30}/I_0	0.341	0.343	0.064	0.199
I_{60}/I_0	0.042	-0.053	-0.034	0.022
Kadowaki model	0.686 ***	0.533 **	0.755 ***	0.681 ***
$\log(I_0)$	0.544 **	0.262	0.75 ***	0.593 **
$\log(I_{120})$	0.339	0.358	0.509 *	0.507 *
$\log(I_{30})$	0.642 **	0.488 *	0.684 ***	0.634 **
$\log(I_{60})$	0.411	0.204	0.481 *	0.359
$\log(I_{90})$	0.339	0.11	0.461 *	0.335
CPI_0	0.516 *	0.269	0.677 ***	0.464 *
CPI_{120}	0.5 *	0.321	0.252	0.185

first-phase Stumvoll	0.757 ***	0.545 **	0.831 ***	0.706 ***
second-phase Stumvoll	0.544 **	0.345	0.758 ***	0.665 ***

The lowest significant correlation with GLP-1 stimulated insulin secretion measurements was found for I_{120}/G_{120} and IGI_{30}.

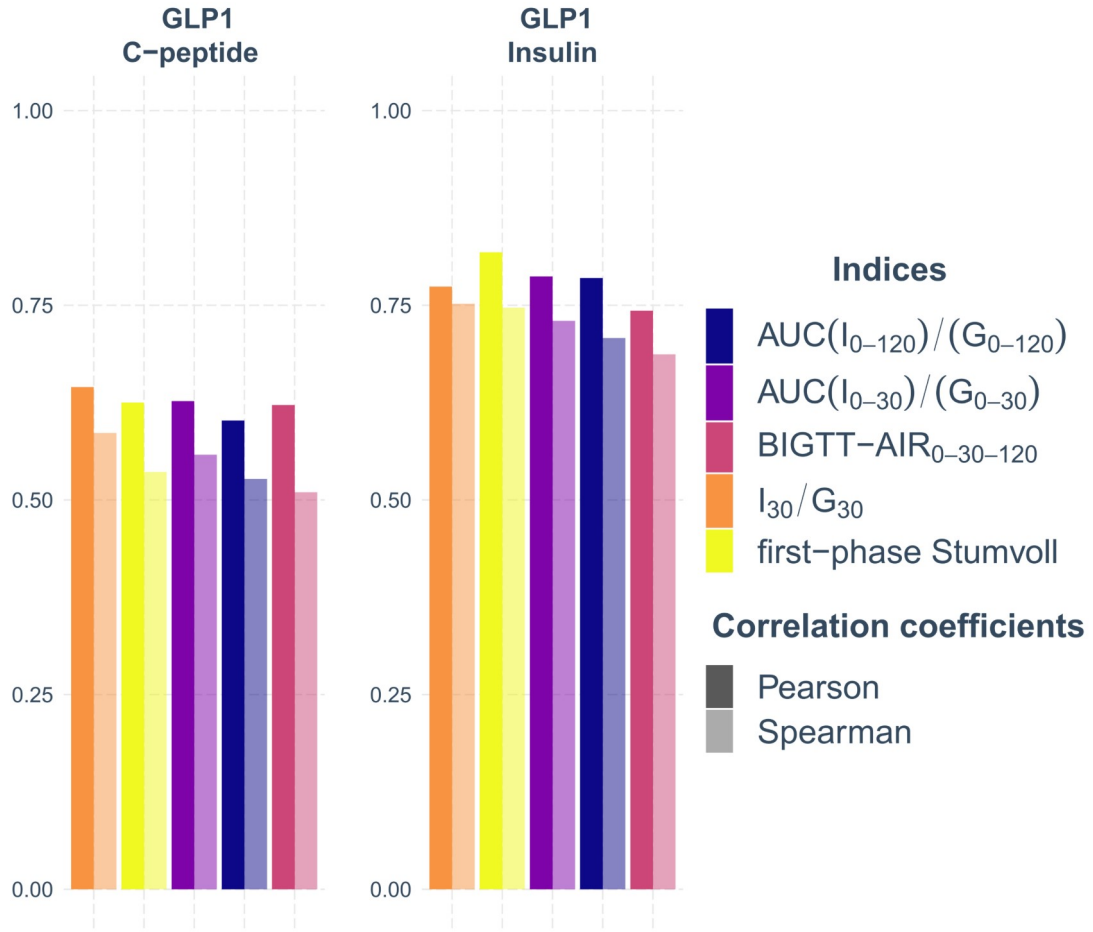


Figure 11: Indices with the highest Spearman's and Pearson's correlation coefficients in a group of 76 participants compared to GLP-1 stimulated insulin secretion during hyperglycaemic clamp. Adapted from Katsiaryna Prystupa et al. 2022.

The investigation of the linear relationship between GLP-1-stimulated clamp-derived insulin secretion and surrogate indices based on OGTT measurements in the entire cohort yielded the following 5 indices as having the highest correlation coefficients: the I_{30}/G_{30}, first-phase Stumvoll, AUC ($I_{0\text{-}120}$)/AUC ($G_{0\text{-}120}$), AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), and BIGTT-$AIR_{0\text{-}60\text{-}120}$ are strongly associated with the incretin stimulated insulin release (Figure 11). The weakest correlation coefficients have CIR_{120} and CPI_{120}.

4 Discussion

Parts of the data were published (Katsiaryna Prystupa et al. 2022).

The reliable and practical assessment of insulin secretion remains a major challenge in diabetes research and clinical care. Impaired insulin secretion is a central pathophysiological feature of both prediabetes and type 2 diabetes, and its early detection is critical for identifying individuals at risk, understanding disease heterogeneity, and guiding therapy.

Gold-standard methods for quantifying insulin secretion, such as the intravenous glucose tolerance test (IVGTT) and the hyperglycemic clamp, provide precise functional measurements by delivering glucose parenterally. However, these gold-standard methods are highly time-consuming and expensive, excluding their use in clinical practice and limiting their application in scientific research.

In contrast, surrogate indices derived from the oral glucose tolerance test (OGTT) offer a practical, and cost-effective alternative for estimating beta-cell function in both clinical and research settings. Unlike intravenous glucose administration, the OGTT mimics physiological nutrient intake and stimulates insulin secretion not only via direct glucose sensing in pancreatic beta cells but also through the release of incretin hormones, particularly glucagon-like peptide 1 (GLP-1). This incretin effect enhances glucose-dependent insulin secretion and plays a key role in physiological postprandial glucose regulation.

While the inclusion of the incretin axis introduces additional physiological complexity, it also represents a potential advantage. The ability of the OGTT to capture both glucose-triggered and incretin-mediated insulin responses provides more comprehensive insights into beta-cell function under real-life metabolic conditions. Consequently, OGTT-derived indices may be useful not only for assessing glucose-dependent insulin secretion capacity but also for reflecting GLP-1–mediated responses.

Over the past decades, numerous surrogate indices of insulin secretion derived from OGTT have been proposed and used in various studies. Some of them have demonstrated strong correlations with gold standard methods in selected

populations (Hanson et al. 2000; Retnakaran et al. 2008; Tura, Kautzky-Willer, and Pacini 2006). However, these indices have not been systematically compared and validated in a large, well-characterized cohort that includes individuals with a diverse spectrum of glucose tolerance. Moreover, their ability to capture GLP-1-stimulated insulin secretion has not been systematically investigated. This represents a major knowledge gap—particularly given the growing interest in the heterogeneity of diabetes and prediabetes, and the pivotal role of the incretin axis in both the pathophysiological characterization and therapeutic stratification of metabolic diseases.

This study aimed to fill this gap by systematically evaluating a broad range of previously published OGTT-derived surrogate indices in two independent, deeply phenotyped cohorts (n=316 and n=76). The first objective was to identify which indices most accurately reflect insulin secretion as measured by IVGTT and hyperglycaemic clamp, independent of glucose tolerance status. The second objective was to determine whether any of these indices also correlate with GLP-1–stimulated insulin secretion, assessed using a modified hyperglycaemic clamp protocol that included a standardized GLP-1 infusion.

To achieve robust results and to recognize potential deviations from a normal distribution, correlations between simple and gold-standard measures of insulin secretion were determined using both Pearson's and Spearman's correlation coefficients in two independent cohorts. Here, it is important to note that the correlation coefficients obtained through the Pearson's test, which detects a linear relationship between variables, may not always match those obtained through the Spearman test. The disparities in correlation coefficients observed between the two methods for certain surrogate indices can be most probably attributed to non-normal distributions some of these variables.

The overall highest correlation coefficients obtained with both Pearson's and Spearman's tests were found for AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), I_{30}/G_{30}, first-phase Stumvoll and Kadowaki model. These indices consistently showed high correlation coefficients with gold standard measures, obtained from C-peptide and insulin measurements in two phases for both NGT and prediabetes groups, reflecting their robustness in estimating insulin secretion dynamics.

These four indices, which are constructed from snapshots of insulin and glucose levels at critical early time points of oral glucose stimulated insulin secretion (at fasting and at 30 minutes after stimulation), are particularly effective in capturing the rapid insulin response. This is highly relevant for detecting early beta cell dysfunction in individuals at risk for developing diabetes before more obvious symptoms of glucose intolerance appear. As robust surrogate indices, they may help predict diabetes in non-diabetic individuals and are sufficient for both clinical and research application.

In contrast, indices that use measurements at longer time intervals after oral glucose stimulation, e.g., CP_{120}, CIR_{120}, and IGI_{120} showed weaker correlation with gold-standard measures. This suggests that they may be influenced by secondary physiological processes. These sources of bias comprise physiologic processes such as incretin hormone action and insulin clearance.

One key factor contributing to this difference is incretin-mediated insulin secretion. Incretin hormones, predominantly GLP-1, potentiate insulin secretion in response to oral glucose load. However, the incretin effect is not uniform, its effect is generally less pronounced in the early phase of glucose challenge and might become more dominant at later time points (Herzberg-Schäfer et al. 2010). Consequently, indices that rely on early phase insulin secretion may better capture direct beta cell responsiveness, whereas indices incorporating later time points (e.g. 120-minutes) may be more influenced by the incretin response.

Another major determinant of time-depending insulin dynamics is insulin clearance. Insulin clearance reflects the elimination of insulin from the circulation, which can amount to more than 50% by first pass through the liver (Wallace, Levy, and Matthews 2004), which can significantly influence insulin-based indices. As a result, later insulin measurements may be more affected by individual variability in insulin clearance, further contributing to the weaker correlations observed for 120-minute indices.

Notably, C-peptide measurements provide a distinct advantage in this context, as C-peptide is not eliminated by the liver (Marques, Fontaine, and Rogers 2004). Unlike insulin, C-peptide is predominantly cleared by the kidneys and therefore C-peptide-based indices not biased by insulin clearance (Marques,

Fontaine, and Rogers 2004). Instead, C-peptide measurements could be biased by the relatively long half-life of approximately 30 minutes of C-peptide (Wahren and Larsson 2015), which may smooth out rapid fluctuations in insulin response.

Overall, the findings suggest that insulin-based indices correlate more strongly with gold-standard measures compared to C-peptide-based indices, aligning with previous findings (Tura, Kautzky-Willer, and Pacini 2006). The early-phase indices (e.g. AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), I_{30}/G_{30}, first-phase Stumvoll and Kadowaki model) provide a more direct assessment of beta cell responsiveness, whereas later-phase indices are susceptible to confounding effect from incretin action.

Indeed, after examining potential surrogate markers of GLP-1-stimulated insulin secretion, strong correlations were found for AUC ($I_{0\text{-}120}$)/AUC ($G_{0\text{-}120}$), and BIGTT-$AIR_{0\text{-}60\text{-}120}$ during the modified hyperinsulinemic clamp, where GLP-1 was administered as a bolus injection, followed by a continuous infusion. These findings align with the hypothesis that longer assessment periods during an OGTT, are necessary to fully capture incretin-mediated on insulin secretion dynamics (Herzberg-Schäfer et al. 2010).

Incretins play a pivotal role in augmenting postprandial insulin release. Mimicking incretin action or increasing incretin levels have been proven to be highly efficacious therapeutic interventions in type 2 diabetes management (e.g. GLP-1 receptor agonists). Moreover, the strong correlation of early-phase indices (I_{30}/G_{30}, AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), first-phase Stumvoll) with GLP-1-stimulated insulin secretion underscores their effectiveness in capturing insulin secretion enhancements due to early incretin release. During the first 30 minutes after glucose ingestion—when incretin hormones are most active—these indices capture the peak insulin response. This response includes both the insulin directly secreted by the pancreas in reaction to glucose and the additional secretion driven by the incretins (Nauck and Meier 2018).

Given the increasing clinical use of incretin-based therapies, the precise selection of OGTT-derived indices may provide insights into treatment responsiveness. Indices, having the strongest correlation with clamp based GLP-1-stimulated insulin secretion, may serve as valuable tool for evaluating the efficacy of GLP-1

receptor agonist, identifying which patients are most likely benefit from this treatment. In clinical practice, such surrogate indices could help in stratifying individuals who are more incretin-responsive and therefore more suitable candidates for therapies targeting the incretin axis. This could support personalized treatment decisions and improve therapeutic outcomes.

The limitation of this study is the lack of ethnic diversity among the participants, which limits the generalisation of the findings. The indices evaluated may not perform uniformly across different ethnic groups. For instance, individuals of Asian descent often exhibit lower beta cell function (Gujral et al. 2014; Tura, Kautzky-Willer, and Pacini 2006; Wallace, Levy, and Matthews 2004; Marques, Fontaine, and Rogers 2004). Individuals of African descent may respond differently due to their tendency towards beta cell hyperresponsiveness and reduced insulin clearance (Armiyaw et al. 2020). This variation in beta cell activity and insulin dynamics across ethnicities indicates that the findings from this study may require further validation in ethnically diverse populations to ensure their broader applicability.

Additionally, the relatively small number of participants with type 2 diabetes limits the conclusions that can be drawn for this specific group and highlights the need for further studies to explore these relationships in larger diabetic populations. Despite these points, this study provides valuable insights that can serve as a foundation for more extensive, diverse future studies.

Future research should focus on validating these findings in multiethnic populations and across broader metabolic phenotypes, including obesity, insulin resistance, and established type 2 diabetes. Longitudinal studies could determine whether these indices can predict disease progression or treatment response. Further refinement of these indices could also facilitate their broader implementation in clinical risk assessment tools and trial endpoints.

Taken together, this study provides a systematic evaluation of a wide range of fasting- and OGTT-derived surrogate indices and identifies AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), I_{30}/G_{30}, first-phase Stumvoll and Kadowaki model as the most accurat markers of insulin secretion based on their strong correlation with IVGTT and hyperglycaemic clamp measurements.

In addition to validating these indices, this study also provides novel insights into their ability to reflect GLP-1–stimulated insulin secretion. A subset of indices (I_{30}/G_{30}, first-phase Stumvoll, AUC ($I_{0\text{-}120}$)/AUC ($G_{0\text{-}120}$), AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), and BIGTT-$AIR_{0\text{-}60\text{-}120}$) were found to correlate with clamp-derived measurements of incretin-stimulated insulin release, suggesting their potential use as surrogate markers of incretin responsiveness. The robustness of these findings was supported by consistent results using both Pearson and Spearman correlation analyses, accounting for potential non-normality in index distributions. These indices have shown consistent results across different categories of glucose tolerance and are thus suited for assessing beta-cell function in research and clinical settings.

Given the importance of insulin secretion in the pathophysiology of diabetes, these validated indices offer valuable tool for stratifying individuals by beta cell function, evaluating response to intervention and monitoring disease progression in individuals at elevated risk for type 2 diabetes. Their application is particularly relevant for the classification of patients in novel subgroups of prediabetes (Wagner et al. 2021) and diabetes (Ahlqvist et al. 2018), also for potential therapeutic considerations (Fritsche et al. 2020).

Ultimately, refining these indices could improve diabetes research and clinical decision-making by bridging the gap between physiological data and real-world applications in the treatment of metabolic diseases.

5 Summary

Type 2 diabetes is a disease defined by hyperglycaemia, resulting from a combination of impaired insulin action and impaired insulin secretion. The heterogeneity of type 2 diabetes is secondary to different extents of its underlying components with insulin secretion, due to a dysfunction or decline of beta cells in the Langerhans islets of the pancreas, being the most important factor. Therefore, measuring insulin secretion is crucial to understand the heterogeneity of type 2 diabetes, but also the metabolic state preceding its manifestation, called prediabetes. Recently, many studies have been conducted to identify subgroups of diabetes and prediabetes.

Accurate measurement of insulin secretion requires laborious studies such as performing of a hyperglycaemic clamp or an intravenous glucose tolerance test (IVGTT). Since performing these studies is not feasible in large cohorts and in clinical routine, assessment of simpler surrogate measures is necessary.

This study undertook a rigorous validation of multiple previously introduced indices derived from fasting conditions and oral glucose tolerance tests, comparing them against gold-standard measures such as hyperglycaemic clamp and IVGTT. The hyperglycaemic clamp approach also comprised a glucagon-like peptide 1 (GLP-1) infusion, allowing assessment of a specific aspect of insulin secretion.

The surrogate measures used for comparing insulin secretion from gold-standard measurements were derived from fasting measurements of glucose, insulin, and C-peptide or from measurements of these analytes during oral glucose tolerance test (OGTT). Given that these analytes were assessed at five points of the OGTT (fasting, 30, 60, 90, 120 minutes), this enabled computation of most published surrogate indices of insulin secretion at reasonably high sampling rate. To describe the correlation of gold-standard measures with surrogates, Pearson's and Spearman's correlation coefficients were used. Insulin- and C-peptide-based indices were analysed separately in different subgroups of glucose tolerance in

the Tübingen Family Study. As glucose metabolism deteriorates from normal to impaired fasting glucose, impaired glucose tolerance and ultimately to diabetes, beta cell function declines. Therefore, comparing surrogate indices for beta cell function assessment in different groups according to glucose tolerance can provide insights into the ability of indices to predict glucose tolerance and progression to type 2 diabetes. Moreover, this is important as it provides a better understanding of how different indices of insulin secretion perform in different clinical contexts.

The findings highlighted that most surrogate indices demonstrated significant and robust correlations with gold-standard assessments. Notably, indices such as AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$), I_{30}/G_{30}, first-phase Stumvoll and Kadowaki model showed exceptionally high correlations ($r > 0.6$, $p < 0.05$) across both insulin and C-peptide measurements, affirming their reliability. These indices have high correlation coefficients with measures obtained from both insulin and C-peptide levels from IVGTT and hyperglycaemic clamp.

The strongest association with GLP-1-stimulated insulin secretion was seen for AUC ($I_{0\text{-}120}$)/AUC ($G_{0\text{-}120}$), BIGTT-$AIR_{0\text{-}60\text{-}120}$, I_{30}/G_{30}, first-phase Stumvoll and AUC ($I_{0\text{-}30}$)/AUC ($G_{0\text{-}30}$). By demonstrating that these indices can reliably reflect the physiological effects of GLP-1 on insulin secretion, the study advances our understanding of how OGTT can be used for more nuanced assessments of beta cell function in response to GLP-1.

The study provides a comprehensive assessment of multiple surrogate markers of insulin secretion against gold standard measures, thereby providing a valuable resource for selecting optimum biomarkers of insulin secretion in clinical studies. This will support a more precise assessment of insulin secretion to further our understanding of the heterogeneity in prediabetes and type 2 diabetes.

6 Zusammenfassung

Typ-2-Diabetes ist eine durch Hyperglykämie definierte Krankheit, die aus einer Kombination von beeinträchtigter Insulinwirkung und Insulinsekretion resultiert. Die Heterogenität des Typ-2-Diabetes wird hauptsächlich durch eine Dysfunktion oder einen Rückgang der Betazellen in den Langerhans-Inseln des Pankreas bestimmt. Daher ist die Messung der Insulinsekretion entscheidend, um die Heterogenität des Typ-2-Diabetes zu verstehen sowie den metabolischen Zustand vor seiner Manifestation, der als Prädiabetes bezeichnet wird. Viele Studien wurden durchgeführt, um Subgruppen von Diabetes und Prädiabetes zu identifizieren. Da die Insulinsekretion eine Schlüsselrolle beim Verständnis der Heterogenität von Diabetes und Prädiabetes spielt, erfordert die Identifizierung von Subgruppen zuverlässige Surrogatindizes für die Betazellfunktion.

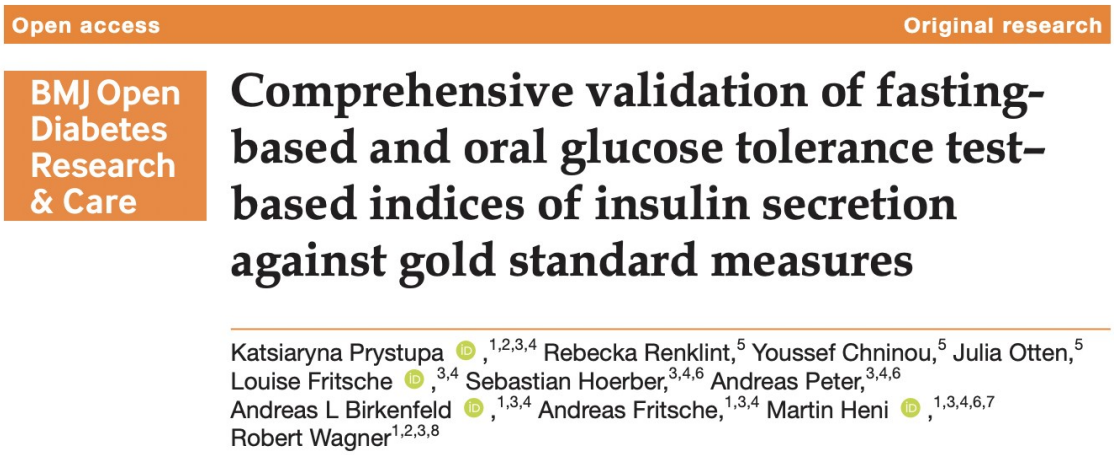
Eine genaue Messung der Insulinsekretion erfordert aufwändige Untersuchungen wie die Durchführung eines hyperglykämischen Clamps oder eines intravenösen Glukosetoleranztests (IVGTT). Da die Durchführung dieser Untersuchungen in großen Kohorten und in der klinischen Routine nicht machbar bzw. sehr aufwändig ist, ist der Einsatz einfacherer Surrogatindizes notwendig.

In dieser Studie wurden mehrere, früher eingeführte Indizes, die aus Nüchternblutproben und oralen Glukosetoleranztests (OGTT) abgeleitet wurden, validiert und mit Goldstandard-Methoden wie dem hyperglykämischen Clamp und dem IVGTT verglichen. In der vorliegenden Studie umfasste der Ansatz des hyperglykämischen Clamps auch eine Glucagon-Like Peptide-1 (GLP-1)-Infusion, die eine Bewertung eines spezifischen Aspekts der Insulinsekretion ermöglichte. Um die Korrelation von Goldstandard-Messungen mit Surrogatindizes zu beschreiben, wurden die Korrelationskoeffizienten nach Pearson und Spearman verwendet. Die Indizes wurden separat in verschiedenen Untergruppen der Glukosetoleranz und in der „Tübingen Family Study“ analysiert. Mit der Verschlechterung des Glukosestoffwechsels von normaler zu beeinträchtigter Nüchternglukose, beeinträchtigter Glukosetoleranz und letztendlich zu Diabetes, nimmt die Betazellfunktion ab. Daher kann der Vergleich

von Surrogatindizes zur Bewertung der Betazellfunktion in verschiedenen Gruppen nach Glukosetoleranz Einblicke in die Fähigkeit der Indizes geben, die Glukosetoleranz und die Progression zum Typ-2-Diabetes vorherzusagen. Darüber hinaus ergibt sich daraus ein besseres Verständnis für die Aussagekraft der verschiedenen Indizes der Insulinsekretion in unterschiedlichen klinischen Kontexten.

Die Ergebnisse zeigten, dass die meisten Surrogatindizes signifikante und robuste Korrelationen mit den Goldstandard-Messungen aufwiesen. Insbesondere Indizes wie AUC (I_{0-30})/AUC (G_{0-30}), I_{30}/G_{30}, erste Phase Stumvoll und Kadowaki-Modell zeigten außergewöhnlich hohe Korrelationen ($r > 0,6$, $p < 0,05$) sowohl bei Insulin- als auch bei C-Peptid-Messungen und bestätigten damit ihre Zuverlässigkeit. Diese Indizes haben hohe Korrelationskoeffizienten mit Variablen, die sowohl aus Insulin- als auch aus C-Peptid-Werten von IVGTT und hyperglykämischem Clamp gewonnen wurden. Die stärkste Assoziation mit GLP-1-stimulierter Insulinsekretion wurde für AUC (I_{0-120})/AUC (G_{0-120}), BIGTT-$AIR_{0-60-120}$, I_{30}/G_{30}, erste Phase Stumvoll und AUC (I_{0-30})/AUC (G_{0-30}) beobachtet. Diese Indizes spiegeln die physiologischen Effekte von GLP-1 auf die Insulinsekretion zuverlässig wider. Damit erweitert die Studie unser Verständnis dafür, wie der OGTT für nuanciertere Bewertungen der Betazellfunktion in Reaktion auf GLP-1 verwendet werden kann. Die Studie erlaubt eine umfassende Bewertung mehrerer Surrogatindizes der Insulinsekretion gegenüber Goldstandard-Methoden und bietet damit eine wertvolle Grundlage für die Auswahl optimaler Biomarker der Insulinsekretion in klinischen Studien. Dies unterstützt eine präzisere Bewertung der Insulinsekretion zur verfeinerten Beschreibung der Heterogenität des Prädiabetes und des Typ-2-Diabetes.

7 Publication



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AUTHOR CONTRIBUTIONS

KP, RW and MH designed the analysis strategy, supervised the project and wrote the manuscript. RR, YC, JO, AB, AP, and AF contributed to the interpretation of the data and edited the manuscript.

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9 Declaration

I hereby declare that I have independently written my dissertation titled "Comprehensive validation of fasting- and oral glucose tolerance test-based indices of insulin secretion against gold-standard measures," under the guidance of Professor Wagner at the University Hospital Tübingen and German Diabetes Center Düsseldorf. My work, which included the study's conception, data analysis, and manuscript preparation, was conducted without unauthorized assistance. I have only used the sources and aids indicated, clearly citing any content from other works.

The dissertation is based on pre-existing dataset from the Tübingen Family Study (TUEF). I did not participate in the recruitment of participants or the collection of primary data.

This dissertation, submitted for a doctorate, has not been part of any other doctoral procedure. The publication in BMJ Open Diabetes Research & Care on 13 September 2022 is based on this work, distinguishing my contribution and that of my co-authors. In addition to the publication in BMJ Open Diabetes Research & Care, an earlier version of this work was made available on medRxiv, facilitating early dissemination and feedback from the scientific community.

I affirm that the statements are true to my knowledge and belief, and I have not omitted any information.

_____ _____

Date Signature Katsiaryna Prystupa

10 Acknowledgments

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11Supplementary: R-code

compare oGTT based secretion indexes with HC and IVGTT

```
library(openxlsx)
library(tidyverse)

library(zoo)

dta <- read.xlsx("dta.xlsx", detectDates = T)
allhoma2 <- read.xlsx("allhoma2.xlsx", detectDates = T)

# Renaming variables, reshaping data, and calculating AUCs
aucs <- dta %>%
    rename_at(vars(INS0:INS120),~str_replace(.,"INS","I")) %>%  # Rename 'INS' columns to 'I'
    rename_at(vars(BZN0:BZ120),~str_replace(.,"(BZN)|(BZ)","G")) %>%  # Rename 'BZN' columns
to 'G'
    rename_at(vars(CPEPT0S:CPEPT120S),~str_remove(str_replace(.,"CPEPT","CP"),"(?<=0)S"))
%>%  # Rename 'CPEPT' columns to 'CP' and remove 'S' at the end if present
    select(NR,date,matches("^(G|I|CP)[0-9]+")) %>%  # Select columns based on pattern
    gather(var,val,-NR,-date) %>%  # Convert from wide to long format
    mutate(analyte = str_extract(var,"(G|I|CP)"),  # Extract the analyte type (G, I, or CP)
           time = as.numeric(str_extract(var,"[0-9]+"))) %>%  # Convert time points to numeric
    select(-var) %>%  # Remove the 'var' column
    filter(!is.na(val)) %>%  # Filter out missing values
    group_by(NR,date,analyte) %>%  # Group data by NR, date, and analyte
    arrange(time) %>%  # Arrange the data by time
    summarise(
      AUC_all = sum(diff(time)*rollmean(val,2)),  # Calculate the overall AUC
      # Note: The following line is duplicated and should probably be corrected or removed
      AUC_0_120 = sum(diff(time[time %in% c(0,120)])*rollmean(val[time %in% c(0,120)],2)),  #
Calculate AUC for 0 to 120 minutes
      AUC_0_30 = sum(diff(time[time %in% c(0,30)])*rollmean(val[time %in% c(0,30)],2)),  #
Calculate AUC for 0 to 30 minutes
      AUC_0_60 = sum(diff(time[time %in% c(0,60)])*rollmean(val[time %in% c(0,60)],2))  # Calculate
AUC for 0 to 60 minutes
    ) %>%
    gather(var,val,starts_with("AUC")) %>%  # Gather AUC variables into long format
```

```
    mutate(variable = paste0(analyte,"_",var)) %>%  # Create a new variable by combining analyte
type and AUC
    select(-var,-analyte) %>%  # Remove the 'var' and 'analyte' columns
    spread(variable,val)  # Spread the data back to wide format with new AUC variables

# List of variables that are required to be positive values
vars_forced_positive = c("IGI_30","IGI_120","Stumvoll_first","Stumvoll_second","CIR_120")

# Define a function to replace zeros with NA in specified variables
zero_to_NA <- function(df, ...) {
     vars_to_clean <- quos(...)  # Use quosures to capture variable names
     temp <- df %>%
          mutate_at(vars(!!!vars_to_clean),~ifelse(.==0,NA,.))  # Replace zeros with NAs
     return(temp)
}

# Begin data transformation and analysis
dta_secvars <- dta %>%
    # Renaming columns: Replace 'INS' with 'I' for insulin-related columns
    rename_at(vars(INS0:INS120), ~str_replace(.,"INS","I")) %>%
    # Renaming columns: Replace 'BZN' or 'BZ' with 'G' for glucose-related columns
    rename_at(vars(BZN0:BZ120), ~str_replace(.,"(BZN)|(BZ)","G")) %>%
    # Renaming columns: Replace 'CPEPT' with 'CP' and remove 'S' after '0' for C-peptide columns
    rename_at(vars(CPEPT0S:CPEPT120S), ~str_remove(str_replace(.,"CPEPT","CP"),"(?<=0)S"))
%>%
    # Joining with another dataset 'aucs'
    left_join(aucs) %>%
    # Transforming and calculating new variables based on existing data
    transmute(NR, date, SEX, gly.cat,
          # Calculating Insulinogenic index (IGI) at different time points
          IGI_30 = (I30-I0)/(G30-G0),
          IGI_60 =(I60-I0)/(G60-G0),
          IGI_120 = (I120-I0) / (G120-G0),
          # Calculating BIGTT-AIR using given formula at different time intervals
          BIGTT_AIR_0_30_120 = exp(8.20 + (0.00178 * I0) + (0.00168 * I30)
                         - (0.000383 * I120) - (0.314 * G0)-(0.109 * G30)
                         + (0.0781 * G120) + (0.180 * as.numeric(SEX))  + (0.032 * BMI)),
          BIGTT_AIR_0_60_120 = exp(8.19 + (0.00339 * I0) + (0.00152 * I60) -(0.000959 * I120)
```

```
                -(0.389 * G0) - (0.142 * G60) + (0.164 * G120) +
                (0.256 * as.numeric(SEX)) + (0.038 * BMI)),
  # Calculating AUC ratios for insulin and glucose at different intervals
  AUC_I0_120_over_G0_120 = I_AUC_all/G_AUC_all,
  AUC_I0_30_over_G0_30 = I_AUC_0_30/G_AUC_0_30,
  AUC_I0_60_over_G0_60 = I_AUC_0_60/G_AUC_0_60,
  AUC_CP_0_30_over_G0_30 = CP_AUC_0_30/G_AUC_0_30,
  AUC_CP_0_60_over_G0_60 = CP_AUC_0_60/G_AUC_0_60,

  AUC_CP_0_120_over_G0_120 = CP_AUC_all/G_AUC_all,
  # AUC calculations for insulin and C-peptide
  AUC_I_all = I_AUC_all,
  AUC_CP_all = CP_AUC_all,
  # Calculating C-peptide to Glucose ratio (CIR) at different time points
  CIR_120 = I120/(G120*(G120-3.89)),
  CIR_60 = I60/(G60*(G60-3.89)),
  CIR_30 = I30/(G30*(G30-3.89)),
  # Ratios of insulin to glucose at different time points
  I0_over_G0 = I0/G0,
  I30_over_G3O = I30/G30,
  I60_over_G60 = I60/G60,
  I120_over_G120 = I120/G120,
  # Ratios of C-peptide to glucose at different time points
  CP0_over_G0 = CP0/G0,
  CP30_over_G30 = CP30/G30,
  CP60_over_G60 = CP60/G60,
  CP120_over_G120 = CP120/G120,
  # Ratios of insulin and C-peptide at different time points
  Insulin_ratio_30 = I30/I0,
  Insulin_ratio_60 = I60/I0,
  Insulin_ratio_120 = I120/I0,
  CP_ratio_30 = CP30/CP0,
  CP_ratio_60 = CP60/CP0,
  CP_ratio_120 = CP120/CP0,
  # Differences in insulin levels at different time points
  I30_minus_I0 = I30-I0,
  I60_minus_I0 = I60-I0,
```

```
            # Original data columns
            I0, I30, I60, I120, CP0, CP30, CP60, CP120,
            # Calculating Stumvoll indices
            Stumvoll_first = 1283+(1.829 * I30) -(138.7 * G30) +(3.772 * I0),
            Stumvoll_second = 287+(0.4164 * I30)-(26-07 * G30) +(0.9226 * I0),
            # Kadowaki model calculation
            Kadowaki_model = (I30-I0)/G30,
            # Calculating OGTT-CPI at different time points
            OGTT_CPI_0 = 100*CP0*0.0030/(G0*18),
            OGTT_CPI_120 = 100*CP120*0.0030/(G120*18),
            # Logarithmic transformations of insulin levels
            Log_I0 = log(I0),
            Log_I30 = log(I30),
            Log_I60 = log(I60),
            Log_I90 = log(I90),
            Log_I120 = log(I120),
            # HOMA-B calculation
            HOMA_B = 20*I0/6.9/(G0-3.5)

            ) %>%
            # Handling negative values
            mutate_at(vars(vars_forced_positive), ~ifelse(.<0, NA, .)) %>%
            # Joining with another dataset 'allhoma2'
            left_join(allhoma2) %>%
            # Converting zeros to NA in specific columns
            zero_to_NA(AUC_CP_0_30_over_G0_30, AUC_CP_0_120_over_G0_120)

# Using the 'naniar' library to summarize missing variables
library(naniar)
miss_var_summary(dta_secvars)
```

Loading and Analyzing Missing Data in the Botnia Dataset

```
# Load Botnia data
botdat0 <- read.xlsx("Botnia-OGTT_031220.xlsx", detectDates = T) %>%
    # Selecting specific columns: NR, botdate, and various B-GLU and B-INS, B-CP columns
    select(NR, botdate = 3, `B-GLU__10`:`B-INS_60PM`,
        `B-CP_10S`:`B-CP60S`)
```

```r
# Analyze missing values in the dataset
missings <- botdat0 %>%
        # Reshape the data from wide to long format
        gather(var, val, -NR, -botdate) %>%
        # Grouping by variable to calculate missing values for each variable
        group_by(var) %>%
        # Summarizing the data to get the proportion of missing values per variable
        summarise(missings = sum(is.na(val)/n()))

# Use 'miss_var_summary' from the 'naniar' package to get a detailed summary of missing data
miss_var_summary(botdat0)
```

Imputing Missing Values in the Botnia Dataset

```r
# Load the 'mice' package for multiple imputation
library(mice)

# Prepare the dataset for imputation
botdat_w_miss <- botdat0 %>%
            # Remove 'NR' and 'botdate' columns as they are not needed for imputation
            select(-NR, -botdate)
# Store original column names for later use
oldnames <- names(botdat_w_miss)

# Adjust column names to be syntactically valid (removes problematic characters)
names(botdat_w_miss) <- make.names(names(botdat_w_miss))

# Set seed for reproducibility of the imputation
set.seed(1975)

# Perform multiple imputation using the 'mice' function
completer <- mice(botdat_w_miss)

# Extract the completed data (first imputed dataset by default)
botdat_completed <- complete(completer)

# Reassign original column names to the completed dataset
names(botdat_completed) <- oldnames
```

Combine and Transform Data for Analysis

```
# Binding the original data with the completed imputed data
botdat <- bind_cols(botdat0 %>% select(NR, botdate), botdat_completed) %>%
    # Reshaping the data from wide to long format
    gather(var, val, -NR, -botdate) %>%
    # Extracting 'analyte' and 'time' from the variable names
    mutate(analyte = str_extract(var, "(INS|GLU|CP)"),
           time = str_extract(var, "[0-9]+"),
           # Converting time to negative for specific conditions, otherwise, keeping it as is
           time_update = ifelse(str_detect(var, "(__)|(CP_[0-9]+S)"),
                                as.numeric(time) * -1,
                                as.numeric(time))) %>%
    # Removing unnecessary columns and filtering out NAs
    select(-time, -var) %>%
    filter(!is.na(val), !is.na(time_update)) %>%
    # Grouping data by NR, botdate, and analyte, then arranging by updated time
    group_by(NR, botdate, analyte) %>%
    arrange(time_update) %>%
    # Calculating baseline and AUC for different time intervals
    summarise(
      baseline10 = mean(val[time_update <= 0], na.rm = T) * 50,
      AUC_10_60 = sum(diff(time_update[time_update >= 10 & time_update <= 60]) *
                        rollmean(val[time_update >= 10 & time_update <= 60], 2)),
      baseline = mean(val[time_update <= 0], na.rm = T) * 10,
      AUC_0_10 = sum(diff(time_update[time_update >= 0 & time_update <= 10]) *
                       rollmean(val[time_update >= 0 & time_update <= 10], 2)))  %>%
    # Calculating incremental AUC (iAUC) for different time intervals
    mutate(iAUC_10_60 = AUC_10_60 - baseline10,
           iAUC_0_10 = AUC_0_10 - baseline) %>%
    # Filtering out negative iAUC values
    filter(iAUC_10_60 >= 0, iAUC_0_10 >= 0) %>%
    # Reshaping the data for further analysis
    gather(variable, value, baseline10:iAUC_0_10) %>%
    # Creating a new variable name combining analyte and the variable
    mutate(varname = paste0(analyte, "_", variable)) %>%
    # Selecting and rearranging columns
    select(-analyte, -variable) %>%
```

```
    # Spreading the data from long to wide format based on the new variable name
    spread(varname, value)
```

Combining and Filtering Data for Analysis

```
# Combining the datasets 'dta_secvars' and 'botdat'
allindices <- dta_secvars %>%
      # Joining with 'botdat' using common keys
      inner_join(botdat) %>%
      # Calculating the absolute difference in days between 'botdate' and 'date'
      mutate(timelag = abs(botdate - date)) %>%
      # Grouping by participant identifier 'NR'
      group_by(NR) %>%
      # Sorting each group by 'timelag' to find the closest date
      arrange(timelag) %>%
      # Filtering to keep only the first (closest) record for each 'NR'
      filter(row_number() == 1) %>%
      # Keeping records where the timelag is less than 365 days
      filter(timelag < 365) %>%
      # Categorizing 'glycemia' based on 'gly.cat' categories
      mutate(glycemia = case_when(
        gly.cat == "NGT" ~ "1_NGT",
        gly.cat %in% c("IFG", "IGT", "IFG+IGT") ~ "2_prediabetes",
        gly.cat == "DIA" ~ "3_diabetes"
      ))

# This code segment combines two datasets, 'dta_secvars' and 'botdat', and then processes them
to:
# 1. Determine the time difference between measurements.
# 2. Filter the closest measurements within a year for each participant.
# 3. Categorize participants based on their glycemic status.
```

Creating and Exporting a Summary Table

```
# Load necessary libraries for table creation and formatting
library(htmlTable)
library(tableone)

# Preparing data for the summary table
tabonedata <- allindices %>%
     # Selecting relevant columns from 'allindices'
```

```
      select(NR, SEX, date, glycemia, timelag, gly.cat) %>%
      # Joining with additional data from 'dta'
      left_join(dta %>% select(NR, date, AGE, BMI, PROCFETT, HBA1C)) %>%
      # Filtering out rows where 'glycemia' is missing
      filter(!is.na(glycemia)) %>%
      # Converting 'timelag' to numeric type
      mutate(timelag = as.numeric(timelag))

# Creating a subset of data for the summary table
sumtab <- tabonedata %>% select(SEX, AGE, BMI, glycemia) %>% ungroup()

# Creating a summary table using the 'tableone' package
t1 <- tableone::CreateTableOne(vars = names(tabonedata)[-1], # Exclude 'NR' from the variables
                               data = tabonedata,
                               factorVars = c("SEX", "glycemia")) # Specifying categorical variables

# Printing the table with specific formatting for non-normal variables
print(t1, nonnoral = c("timelag", "AGE", "BMI", "PROCFETT", "HBA1C"))

# Generating an HTML version of the table
ht <- htmlTable(print(t1, nonnormal = c("timelag", "AGE", "BMI", "PROCFETT", "HBA1C"))

# Creating a file to save the HTML table
f1 <- file("tabl_1.html", encoding = "UTF-8")

# Writing the HTML table to the file
cat(ht, file = f1)

# Closing the file connection
close(f1)

# This code block creates a well-structured summary table from the 'allindices' and 'dta' datasets. It
then formats this table for both normal and non-normal variables and exports it as an HTML file.
```

Computing and Exporting Large Correlation Matrices

```
# Load necessary library for HTML table formatting
library(htmlTable)

# Prepare data for correlation matrix computation
cormtrx0 <- allindices %>%
```

```
        # Selecting relevant variables for correlation analysis
        select(NR, IGI_30:Log_I120, CP_iAUC_0_10, INS_iAUC_0_10, INS_iAUC_10_60,
CP_iAUC_10_60,
               starts_with("homa2"), starts_with("HOMA_"), glycemia) %>%
        # Reshape data for OGTT indices
        gather(OGTT_index, OGTT_secretion, IGI_30:Log_I120, homa2_b_cpep, homa2_b_i,
HOMA_B) %>%
        # Reshape data for IVGTT indices
        gather(IVGTT_index, IVGTT_secretion, CP_iAUC_0_10:CP_iAUC_10_60) %>%
        # Group data by OGTT and IVGTT indices
        group_by(OGTT_index, IVGTT_index) %>%
        # Replace infinite values with NA and filter out extreme outliers
        mutate(OGTT_secretion = replace(OGTT_secretion, OGTT_secretion == Inf, NA)) %>%
        filter(abs(as.numeric(scale(OGTT_secretion))) < 5,
               abs(as.numeric(scale(IVGTT_secretion))) < 5)

# Display frequency of different OGTT indices
table(cormtrx0$OGTT_index)

# Function to format p-values for readability
pformat <- function(pval) {
  ret <- ifelse(pval < 0.001, "<0.001",
              ifelse(pval < 0.05 & pval > 0.01, "<0.05",
                  ifelse(pval < 0.01 & pval > 0.001, "<0.01", "not")))
  return(trimws(ret))
}

# Compute correlation matrices and summarise data
cormtrx <- cormtrx0 %>%
        group_by(OGTT_index, IVGTT_index) %>%
        # Summarize data to compute correlations and p-values
        summarise(N = sum(complete.cases(OGTT_secretion, IVGTT_secretion)),
                  cor_pearson = round(cor(x = OGTT_secretion, y = IVGTT_secretion, use =
"pairwise.complete.obs"), 3),
                  cor_spearman = round(cor(x = OGTT_secretion, y = IVGTT_secretion, use =
"pairwise.complete.obs", method = "spearman"), 3),
                  cor_pvalue = pformat(cor.test(x = OGTT_secretion, y = IVGTT_secretion, use =
"pairwise.complete.obs")$p.value),
```

```
            Sp_pvalue = pformat(cor.test(x = OGTT_secretion, y = IVGTT_secretion, use =
"pairwise.complete.obs", method = "spearman")$p.value))   %>%
        # Arrange data by Pearson correlation
        arrange(desc(cor_pearson))

# Generate HTML table from the correlation matrix
c0 <- htmlTable::htmlTable(cormtrx)

# Create a file to save the HTML table
f1 <- file("IVGTT_OGTT_secretion_correlations_all_v", encoding = "UTF-8")

# Writing the HTML table to the file
cat(c0, file = f1)

# Closing the file connection
close(f1)

# Display the HTML table
c0

# This code block computes correlation matrices between various OGTT and IVGTT indices,
formats the p-values,and exports the result as an HTML file. It includes both Pearson and Spearman
correlations and their respective p-values.
```

Creating a Stratified Correlation Matrix

```
# Preparing the data for stratified correlation analysis
cormtrx_strat <- cormtrx0 %>%
        # Grouping data by OGTT index, IVGTT index, and glycemia categories
        group_by(OGTT_index, IVGTT_index, glycemia) %>%
        # Filtering out rows with missing values in OGTT or IVGTT secretion
        filter(complete.cases(OGTT_secretion, IVGTT_secretion)) %>%
        # Ensuring that there are more than one observation for each group
        filter(n() > 1) %>%
        # Summarizing data to compute correlations and p-values for each group
        summarise(N = n(),
                cor_pearson = round(cor(x = OGTT_secretion, y = IVGTT_secretion, use =
"pairwise.complete.obs"), 3),
                cor_spearman = round(cor(x = OGTT_secretion, y = IVGTT_secretion, use =
"pairwise.complete.obs", method = "spearman"), 3),
```

```
            cor_pvalue = pformat(cor.test(x = OGTT_secretion, y = IVGTT_secretion, use =
"pairwise.complete.obs")$p.value),
            Sp_pvalue = pformat(cor.test(x = OGTT_secretion, y = IVGTT_secretion, use =
"pairwise.complete.obs", method = "spearman")$p.value)) %>%
      # Arranging results by glycemia category and Pearson correlation in descending order
      arrange(glycemia, desc(cor_pearson))

# This code block performs a stratified correlation analysis, grouping the data by glycemia status. It
calculates both Pearson and Spearman correlations along with their p-values for each
group.Results are sorted by glycemia category and the strength of Pearson correlation.
```

Formatting Correlation Values with Significance Indicators

```
# Modifying the stratified correlation matrix with formatted correlation values
stern_IVGTT <- cormtrx_strat %>%
 # Adding significance indicators to Spearman correlations based on p-values
 mutate(Spearman_Cor = case_when(
  Sp_pvalue == "<0.05" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " *"),
  Sp_pvalue == "<0.01" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " **"),
  Sp_pvalue == "<0.001" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " ***"),
  TRUE ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " ")
 )) %>%
 # Adding significance indicators to Pearson correlations based on p-values
 mutate(Pearson_Cor = case_when(
  cor_pvalue == "<0.05" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " *"),
  cor_pvalue == "<0.01" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " **"),
  cor_pvalue == "<0.001" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " ***"),
  TRUE ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " ")
 )) %>%
 # Classifying the phase of IVGTT index
 mutate(phase = case_when(
  IVGTT_index == "CP_iAUC_10_60" | IVGTT_index == "INS_iAUC_10_60" ~ "second",
  TRUE ~ "first"
 ))
# This code block enhances the readability of the correlation matrix by adding asterisks to indicate
the significance level of both Spearman and Pearson correlations. It also classifies the data into
'first' and 'second' phases based on IVGTT indices.
```

Creating and Saving a Formatted Table for NGT First & Second-Phase

```
# Load the kableExtra package for advanced table formatting
library(kableExtra)

# Creating a table for NGT group in the first-phase
kbl_NGT_IVGTT_first <- stern_IVGTT %>%
  # Filtering for NGT glycemia category and the first-phase
  filter(glycemia == "1_NGT" & phase == "first") %>%
  # Selecting relevant columns for the table
  select(row = OGTT_index, column = IVGTT_index, Spearman_Cor, Pearson_Cor) %>%
  # Grouping by OGTT index for pivoting
  group_by(row) %>%
  # Pivoting the data to a wider format for table creation
  pivot_wider(names_from = column, values_from = c(Spearman_Cor, Pearson_Cor)) %>%
  # Arranging rows for better readability
  arrange(row) %>%
  # Creating a kable table
  kbl() %>%
  # Applying classic styling to the table
  kable_classic(full_width = F, html_font = "Cambria") %>%
  # Saving the table as an HTML file
  save_kable(file = "tabl_NGT_IVGTT_first.html", self_contained = T)

# This code block generates a table for the NGT (Normal Glucose Tolerance) category in the first-phase of the study, formats it using kableExtra, and saves it as an HTML file. The table includes both Spearman and Pearson correlation coefficients for various OGTT and IVGTT indices.
```

Creating and Saving a Formatted Table for Prediabetes First-Phase

```
# Reusing the 'kableExtra' package for advanced table formatting (already loaded)

# Creating a table for the prediabetes group in the first-phase
kbl_NGT_IVGTT_first <- stern_IVGTT %>%
  # Filtering for the prediabetes glycemia category and the first-phase
  filter(glycemia == "2_prediabetes" & phase == "first") %>%
  # Selecting relevant columns for the table
  select(row = OGTT_index, column = IVGTT_index, Spearman_Cor, Pearson_Cor) %>%
  # Grouping by OGTT index for pivoting
  group_by(row) %>%
```

```
  # Pivoting the data to a wider format for table creation
  pivot_wider(names_from = column, values_from = c(Spearman_Cor, Pearson_Cor)) %>%
  # Arranging rows for better readability
  arrange(row) %>%
  # Creating a kable table
  kbl() %>%
  # Applying classic styling to the table
  kable_classic(full_width = F, html_font = "Cambria") %>%
  # Saving the table as an HTML file
  save_kable(file = "tabl_prediabetes_IVGTT_first.html", self_contained = T)

# This code block generates a table for the prediabetes group in the first-phase of the study,
formats it using kableExtra, and saves it as an HTML file. The table showcases both Spearman and
Pearson correlation coefficients for various OGTT and IVGTT indices.
```

Creating and Saving a Formatted Table for Diabetes First-Phase

```
# Utilizing 'kableExtra' package for advanced table styling (already loaded)
# Creating a table specifically for the diabetes group in the first-phase
kbl_NGT_IVGTT_first <- stern_IVGTT %>%
  # Filtering the data for the diabetes category and the first-phase
  filter(glycemia == "3_diabetes" & phase == "first") %>%
  # Selecting necessary columns for the table
  select(row = OGTT_index, column = IVGTT_index, Spearman_Cor, Pearson_Cor) %>%
  # Grouping by OGTT index to facilitate data transformation
  group_by(row) %>%
  # Pivoting the data from long to wide format for table display
  pivot_wider(names_from = column, values_from = c(Spearman_Cor, Pearson_Cor)) %>%
  # Arranging the data by OGTT index for better readability
  arrange(row) %>%
  # Creating a table using kable
  kbl() %>%
  # Applying a classic style to the table
  kable_classic(full_width = F, html_font = "Cambria") %>%
  # Saving the table as an HTML file
  save_kable(file = "tabl_diabetes_IVGTT_first.html", self_contained = T)

# This code block generates a table for the diabetes group in the first-phase of the study.
# It is formatted using kableExtra for an improved appearance and is saved as an HTML file.
```

```
# The table includes Spearman and Pearson correlation coefficients for various OGTT and IVGTT indices.
```

Preparing and Arranging IVGTT Correlation Data for Plotting

```
# Filter the data to remove non-significant correlations and focus on the first-phase
IVGTT_plot <- stern_IVGTT %>%
  filter(!cor_pvalue == "not" & !Sp_pvalue == "not") %>%
  mutate(phase = case_when(
    IVGTT_index == "CP_iAUC_10_60" | IVGTT_index == "INS_iAUC_10_60" ~ "second",
    TRUE ~ "first"
  )) %>%
  filter(phase == "first")

# Select and arrange the top correlations for each glycemia category and IVGTT index
IVGTT_Sp_Pr <- IVGTT_plot %>%
  select(row = OGTT_index, column = IVGTT_index, cor_spearman, glycemia, cor_pearson) %>%
  arrange(desc(cor_spearman), desc(cor_pearson)) %>%
  group_by(glycemia, column) %>%
  top_n(3) %>%
  pivot_longer(col = c(cor_pearson, cor_spearman))

## Selecting by cor_pearson

# Load the jtools library for advanced plotting functions
library(jtools)

# Define label mappings for the plot axes and legends
column1<- c("INS_ss_glucose"=" Insulin (HC)",
            "INS_iAUC_0_10"=" Insulin iAUC (IVGTT)",
            "CP_iAUC_0_10"=" C-peptide iAUC (IVGTT)",
            "CPEP_ss_glucose"=" C-peptide (HC)",
            "CP_iAUC_10_60"="C-peptide iAUC (IVGTT) ",
            "INS_iAUC_10_60"="Insulin iAUC (IVGTT) ",
            "INS_ss_fp_glucose"="Insulin (HC)",
            "CPEP_ss_fp_glucose"="C-peptide (HC)",
            "INS_ss_glp1"=" GLP1 Insulin",
            "CPEP_ss_glp1"="GLP1 C-peptide")

# Define label mappings for glycemia categories
```

```
glycemia1 <- c("2_prediabetes" = "Prediabetes",
               "1_NGT" = "NGT",
               "3_diabetes" = "Diabetes")

# Check the frequency of different OGTT indices
table(IVGTT_Sp_Pr$row)
# This code prepares data for visualizing IVGTT correlations, focusing on significant Spearman and Pearson correlations.
# It uses 'pivot_longer' to reshape data for visualization, 'jtools' for advanced plotting capabilities, and defines custom label mappings for clarity in the resulting plots.
```

Defining Readable Labels for Various Indices

```
# Defining Readable Labels for Various Indices

# Create a named vector 'indeces' to map technical index names to more interpretable labels
indeces <- c(
  "Kadowaki_model" = "Kadowaki model",
  "CP_ratio_30" = bquote(.("CP")[30]/"CP"[0]),  # Ratio of C-peptide at time 30 to time 0
  "CP_ratio_60" = bquote(.("CP")[60]/"CP"[0]),  # Ratio of C-peptide at time 60 to time 0
  "CP0_over_G0" = bquote(.("CP")[0]/"G"[0]),    # Ratio of C-peptide to Glucose at time 0
  "CP0" = bquote(.("CP")[0]),                   # C-peptide level at time 0
  "IGI_30" = bquote(.("IGI")[30]),              # Insulinogenic index at 30 minutes
  "OGTT_CPI_0" = bquote(.("CPI")[0]),           # CPI index at time 0 of OGTT
  "Stumvoll_first" = "first-phase Stumvoll",    # First-phase of Stumvoll index
  "AUC_CP_0_120_over_G0_120" = bquote(.("AUC")("CP"[0-120])/("G"[0-120])),  # AUC ratio for CP and Glucose (0-120 minutes)
  "AUC_I0_120_over_G0_120" = bquote(.("AUC")("I"[0-120])/("G"[0-120])),     # AUC ratio for Insulin and Glucose (0-120 minutes)
  "AUC_CP_0_30_over_G0_30" = bquote(.("AUC")("CP"[0-30])/("G"[0-30])),      # AUC ratio for CP and Glucose (0-30 minutes)
  "AUC_CP_0_60_over_G0_60" = bquote(.("AUC")("CP"[0-60])/("G"[0-60])),      # AUC ratio for CP and Glucose (0-60 minutes)
  "AUC_I0_30_over_G0_30" = bquote(.("AUC")("I"[0-30])/("G"[0-30])),         # AUC ratio for Insulin and Glucose (0-30 minutes)
  "CP30_over_G30" = bquote(.("CP")[30]/"G"[30]),                            # Ratio of C-peptide to Glucose at time 30
  "CP60_over_G60" = bquote(.("CP")[60]/"G"[60]),                            # Ratio of C-peptide to Glucose at time 60
```

```
  "I30_over_G3O" = bquote(.("I")[30]/"G"[30]),                    # Ratio of Insulin to Glucose at time
30
  "BIGTT_AIR_0_30_120" = bquote(.("BIGTT-AIR")[0-30-120]),        # BIGTT-AIR index for the
time interval 0-30-120
  "BIGTT_AIR_0_60_120" = bquote(.("BIGTT-AIR")[0-60-120]),        # BIGTT-AIR index for the
time interval 0-60-120
  "CIR_30" = bquote(.("CIR")[30]),                                # C-peptide to Glucose ratio index at time
30
  "CP30" = bquote(.("CP")[30]),                                   # C-peptide level at time 30
  "Insulin_ratio_30" = bquote(.("I")[30]/"I"[0]),                 # Ratio of Insulin levels at time 30 to
time 0
  "Insulin_ratio_60" = bquote(.("I")[60]/"I"[0]),                 # Ratio of Insulin levels at time 60 to
time 0
  "Log_I30" = bquote(.("log")("I"[30])),                          # Logarithm of Insulin level at time 30
  "Log_I0" = bquote(.("log")("I"[0])),                            # Logarithm of Insulin level at time 0
  "I0_over_G0" = bquote(.("I")[0]/"G"[0]),                        # Ratio of Insulin to Glucose at time 0
  "Stumvoll_second" = "second-phase Stumvoll",                    # Second-phase of Stumvoll
index
  "AUC_I_all" = bquote(.("AUC")("I"[all])),                       # AUC for Insulin over all time points
  "AUC_CP_all" = bquote(.("AUC")("CP"[all])),                     # AUC for C-peptide over all time
points
  "I30" = bquote(.("I")[30]),                                     # Insulin level at time 30
  "AUC_I0_30_120_over_G0_30_120" = bquote(.("AUC")("I"[0-120])/("G"[0-120])) # AUC ratio for
Insulin and Glucose (0-120 minutes)
)
# These mappings are essential for creating understandable and visually appealing plots,
translating complex index names into more interpretable and reader-friendly terms.
```

Plotting Correlation Data using ggplot2

```
# Plotting Correlation Data using ggplot2


# Create a bar plot using the prepared IVGTT data
ggplot(IVGTT_Sp_Pr, aes(x = reorder(row, value), y = value, fill = row, group = name, alpha = name))
+
  # Add bar geometry, positioning bars side by side for comparison
  geom_bar(stat = "identity", position = position_dodge()) +
  # Create facets for each combination of 'column' and 'glycemia' categories with customized labels
  facet_wrap(vars(column, glycemia), scales = "free", labeller = labeller(column = column1, glycemia
```

```
= glycemia1)) +
  # Apply a pre-defined nice theme for better aesthetics
  theme_nice() +
  # Customize the alpha scale to distinguish between Pearson and Spearman correlation
coefficients
  scale_alpha_manual("Correlation coefficients", values = c(1, 0.5), labels = c("Pearson",
"Spearman")) +
  # Apply discrete fill colors for different indices with customized labels
  scale_fill_discrete("Indices", labels = indeces) +
  # Customize the x-axis with labels from 'indeces'
  scale_x_discrete(labels = indeces) +
  # Apply several theme settings for improved readability and appearance
  theme(
    legend.text = element_text(size = 20),
    legend.title = element_text(size = 20),
    text = element_text(size = 20),
    title = element_text(size = 17),
    strip.text = element_text(size = 25),
    axis.text.x = element_text(size = 16, angle = 90, vjust = 0.5, hjust = 1)
  ) +
  # Define labels for the y-axis and x-axis
  ylab("") +
  scale_y_continuous("", limits = c(0, 1)) +
  xlab("")

# Save the plot as a PNG file with specified dimensions
ggsave("ivgtt_3top.png", width = 54, height = 35, units = "cm")

# This code block creates a detailed bar plot visualizing the top correlations in IVGTT
data,categorized by different glycemia statuses and indices. It uses facets to allow comparisons
across categories and saves the plot as a high-resolution image.
```

Plotting IVGTT Correlation Data for the Diabetes Group

```
# Filter the data for the diabetes group
NGT_DA <- IVGTT_Sp_Pr %>% filter(glycemia == "3_diabetes")

# Create a bar plot using the filtered data
ggplot(NGT_DA, aes(x = reorder(row, value), y = value, fill = row, group = name, alpha = name)) +
```

```
  # Add bar geometry with dodge position to compare bars side by side
  geom_bar(stat = "identity", position = position_dodge()) +
  # Facet the plot by 'column' and 'glycemia' categories with customized labels
  facet_wrap(vars(column, glycemia), scales = "free", labeller = labeller(column = column1, glycemia
= glycemia1)) +
  # Apply a nice theme for a clean aesthetic
  theme_nice() +
  # Set alpha scale for correlation types (Pearson and Spearman)
  scale_alpha_manual("Correlation coefficients", values = c(1, 0.5), labels = c("Pearson",
"Spearman")) +
  # Set x-axis labels using 'indeces' mapping
  scale_x_discrete(labels = indeces) +
  # Customizing various theme elements for readability
  theme(
    legend.text = element_text(size = 20),
    legend.title = element_text(size = 20),
    text = element_text(size = 20),
    title = element_text(size = 17),
    strip.text = element_text(size = 25),
    axis.text.x = element_text(size = 16, angle = 90, vjust = 0.5, hjust = 1)
  ) +
  # Define y-axis and x-axis labels
  ylab("") +
  scale_y_continuous("", limits = c(0, 1)) +
  xlab("") +
  # Use Brewer color palette for fill scale
  scale_fill_brewer("Indices", labels = indeces, palette = "Set3")

# Save the plot as a PNG file with specified dimensions
ggsave("ivgtt_3top.png", width = 34, height = 25, units = "cm")
# This code block generates a bar plot specifically for the diabetes group, showcasing the top
correlations in IVGTT data. The plot uses a Brewer color palette for distinction and is saved as a
high-resolution image.
```

Processing and Analyzing IVGTT Correlation Data for the Whole Group

```
# Enhancing the correlation matrix with formatted correlation values
stern_IVGTT_b <- cormtrx %>%
  # Adding significance indicators to Spearman correlations based on p-values
```

```
  mutate(Spearman_Cor = case_when(
    Sp_pvalue == "<0.05" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " *"),
    Sp_pvalue == "<0.01" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " **"),
    Sp_pvalue == "<0.001" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " ***"),
    TRUE ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " ")
  )) %>%
  # Adding significance indicators to Pearson correlations based on p-values
  mutate(Pearson_Cor = case_when(
    cor_pvalue == "<0.05" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " *"),
    cor_pvalue == "<0.01" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " **"),
    cor_pvalue == "<0.001" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " ***"),
    TRUE ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " ")
  )) %>%
  # Classifying the phase of IVGTT index
  mutate(phase = case_when(
    IVGTT_index == "CP_iAUC_10_60" | IVGTT_index == "INS_iAUC_10_60" ~ "second",
    TRUE ~ "first"
  )) %>%
  # Filter out non-significant correlations
  filter(!cor_pvalue == "not" & !Sp_pvalue == "not") %>%
  # Focus on the first-phase
  filter(phase == "first")

# Analyzing the top correlations for each IVGTT index
IVGTT_NDv <- stern_IVGTT_b %>%
  select(OGTT_index, IVGTT_index, cor_spearman, cor_pearson) %>%
  arrange(desc(cor_pearson), desc(cor_spearman)) %>%
  group_by(IVGTT_index) %>%
  # Selecting top 8 correlations for each IVGTT index
  top_n(8) %>%
  ungroup() %>%
  group_by(OGTT_index) %>%
  # Determine if there are shared IVGTT indices across OGTT indices
  mutate(shared = n_distinct(IVGTT_index) == n_distinct(.$IVGTT_index),
         cor_spearman = as.numeric(cor_spearman),
         cor_pearson = as.numeric(cor_pearson)) %>%
  filter(shared == TRUE) %>%
```

```
  # Reshape the data for analysis
  pivot_longer(col = c(cor_pearson, cor_spearman))

# This code first enhances the correlation matrix with formatted correlation values and indicators of significance. It then focuses on the first-phase of IVGTT indices and filters out non-significant correlations.
# Lastly, it analyses the top correlations for each IVGTT index, focusing on the most relevant ones.
```

Creating a Multi-Faceted Bar Plot for IVGTT Correlation Data

```
# Generate a bar plot using ggplot2
ggplot(IVGTT_NDv, aes(x = reorder(OGTT_index, value), y = value, fill = OGTT_index, group = name, alpha = name)) +
  # Add bar geometry with dodge position for side-by-side comparison
  geom_bar(stat = "identity", position = position_dodge()) +
  # Create facets for each IVGTT index with free scales on the x-axis and 4 columns
  facet_wrap(vars(IVGTT_index), scales = "free_x", ncol = 4, labeller = labeller(IVGTT_index = column1, glycemia = glycemia1)) +
  # Apply a pre-defined theme for a clean look
  theme_nice() +
  # Customize the alpha scale for different types of correlation coefficients
  scale_alpha_manual("Correlation coefficients", values = c(1, 0.5), labels = c("Pearson", "Spearman")) +
  # Apply discrete fill colors for different OGTT indices
  scale_fill_discrete("Indices", labels = indeces) +
  # Customize the x-axis labels
  scale_x_discrete(labels = indeces) +
  # Customizing various theme elements for aesthetics and readability
  theme(
    legend.text = element_text(size = 17),
    legend.title = element_text(size = 17),
    text = element_text(size = 17),
    title = element_text(size = 17),
    strip.text = element_text(size = 17),
    axis.text = element_text(size = 15),
    axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)
  ) +
  # Define labels for the y-axis and x-axis
  ylab("") +
```

```
  scale_y_continuous("", limits = c(0, 1)) +
  xlab("")

# Save the plot as a PNG file with specified dimensions
ggsave("IVGTT_top_both.png", width = 27, height = 25, units = "cm")

# This code block generates a detailed bar plot showing the top correlations in IVGTT data, categorized by different OGTT indices.The plot is saved as a high-resolution image for presentation or documentation purposes.
```

Analyzing HC Data

```
# Loading necessary libraries
library(lubridate)
# Reading the initial HC data from an Excel file
hyperclamp0 <- read.xlsx("Hyperclamp_Andreas.xlsx", detectDates = TRUE)

# Preprocessing the hyperclamp data
hyperclamp <- read.xlsx("Hyperclamp_Andreas.xlsx", detectDates = TRUE) %>%
  # Creating a new 'NR' column from 'TUEF_ID' and converting 'Studien-Datum' to a date format
  mutate(
    NR = TUEF_ID,
    clampdate = mdy(`Studien-Datum`)
  ) %>%
  # Selecting relevant columns starting with specific prefixes
  select(
    NR, clampdate,
    starts_with("BZ"),
    starts_with("INS", ignore.case = FALSE),
    starts_with("Glukagon"),
    starts_with("CPEP"),
    starts_with("GIR")
  ) %>%
  # Transforming the data from wide to long format
  gather(var, val, -NR, -clampdate) %>%
  # Extracting the analyte name and identifying negative time values
  mutate(
    analyte = str_extract(var, "[A-Za-z]+"),
    negative = str_detect(var, "-[0-9]+"),
```

```
  time = str_extract(var, "[0-9]+")
 ) %>%
 # Converting 'time' to numeric and adjusting for negative values
 mutate(
  time = as.numeric(time) * ifelse(negative, -1, 1)
 ) %>%
 # Dropping unnecessary columns
 select(-var, -negative)

# This code block loads hyperclamp data, processes it by creating necessary columns,and reshapes it for further analysis. It handles date conversions, data transformations, and prepares the dataset for detailed examination.
```

Creating Derived Variables from HC Data

```
# Process hyperclamp data to create derived variables
clampderivates <- hyperclamp %>%
 # Group data by 'analyte' and 'time'
 group_by(analyte, time) %>%
 # Scale values for each group
 mutate(scaledval = as.numeric(scale(val))) %>%
 # Filter out clamps with strong deviation in glucose at steady state (z-score > 3)
 filter(all(abs(scaledval[analyte == "BZ" & time %in% c(100, 110, 120)]) <= 3)) %>%
 # Group data by participant ('NR'), date ('clampdate'), and analyte
 group_by(NR, clampdate, analyte) %>%
 # Summarise data to calculate means for different conditions
 summarise(
  ss_glucose = mean(val[time %in% c(80, 100, 120)], na.rm = TRUE),
  ss_fp_glucose = sum(val[time %in% c(2.5, 5, 7.5, 10)], na.rm = TRUE),
  ss_glp1 = mean(val[time %in% c(160, 170, 180)], na.rm = TRUE),
  ss_arg = mean(val[time > 180], na.rm = TRUE)
 ) %>%
 # Reshape data from wide to long format
 gather(var, val, starts_with("ss_")) %>%
 # Filter out NaN values
 filter(!is.nan(val)) %>%
 # Create new variable names combining 'analyte' and 'var'
 mutate(newname = paste0(analyte, "_", var)) %>%
 # Select and rearrange columns
```

```
  select(-analyte, -var) %>%
  # Spread data from long to wide format based on new variable names
  spread(newname, val)

# This code block processes the hyperclamp data to create new derived variables such as steady-state glucose, fp_glucose, glp1, and arginine levels.
# It involves scaling, grouping, filtering, and reshaping data for analysis.
```

Computing Correlations Between HC Data and Other Variables

```
# Joining hyperclamp data with another dataset and preparing for correlation analysis
clampogtt0 <- dta_secvars %>%
  # Group by participant ID ('NR') and sort by date
  group_by(NR) %>%
  arrange(date) %>%
  # Join with a subset of 'clampderivates'
  left_join(clampderivates %>% select(NR, clampdate, starts_with("INS"), starts_with("CPEP"))) %>%
  # Calculate the time difference between 'clampdate' and 'date'
  mutate(lag = abs(clampdate - date)) %>%
  # Filter records where the difference is less than a year
  filter(lag < 365) %>%
  # Re-group and pick the record with the smallest lag for each participant
  group_by(NR) %>%
  arrange(lag) %>%
  filter(row_number() == 1) %>%
  ungroup() %>%
  # Categorize 'glycemia' based on 'gly.cat'
  mutate(glycemia = case_when(
    gly.cat == "NGT" ~ "1_NGT",
    gly.cat %in% c("IFG", "IGT", "IFG+IGT") ~ "2_prediabetes",
    gly.cat == "DIA" ~ "3_diabetes"
  ))
# Preparing data for correlation matrix calculation
clamp_cormtrx0 <- clampogtt0 %>%
  # Reshape the data from wide to long format for OGTT and clamp indices
  gather(OGTT_index, OGTT_secretion, IGI_30:Log_I120, homa2_b_cpep, homa2_b_i, HOMA_B) %>%
  gather(clamp_index, clamp_secretion, starts_with("CPEP_SS"), starts_with("INS_SS")) %>%
```

```
  # Replace infinite values with NA
  mutate(OGTT_secretion = replace(OGTT_secretion, OGTT_secretion == Inf, NA))

# Calculating the correlation matrix
clamp_cormtrx <- clamp_cormtrx0 %>%
  # Group data by OGTT and clamp indices
  group_by(OGTT_index, clamp_index) %>%
  # Filter out extreme outliers
  filter(abs(as.numeric(scale(OGTT_secretion))) < 5, abs(as.numeric(scale(clamp_secretion))) < 5)
%>%
  # Summarize data to compute correlations and p-values
  summarise(
    N = sum(complete.cases(OGTT_secretion, clamp_secretion)),
    cor_pearson = round(cor(x = OGTT_secretion, y = clamp_secretion, use =
"pairwise.complete.obs"), 3),
    cor_spearman = round(cor(x = OGTT_secretion, y = clamp_secretion, use =
"pairwise.complete.obs", method = "spearman"), 3),
    cor_pvalue = pformat(cor.test(x = OGTT_secretion, y = clamp_secretion, use =
"pairwise.complete.obs")$p.value),
    Sp_pvalue = pformat(cor.test(x = OGTT_secretion, y = clamp_secretion, use =
"pairwise.complete.obs", method = "spearman")$p.value)
  ) %>%
  # Arrange results by Spearman correlation
  arrange(desc(cor_spearman))

# This code block merges hyperclamp data with another dataset, filters records based on time lag,
categorizes glycemia, and computes Pearson and Spearman correlations between various indices.
# The resulting correlation matrix provides insight into the relationships between different
measures.
```

Creating a Descriptive Statistics Table for HC Data

```
# Preparing data for the table
tabonedata <- clampogtt0 %>%
  # Selecting relevant columns from 'clampogtt0'
  select(NR, SEX, gly.cat) %>%
  # Joining with additional data from 'hyperclamp0'
  left_join(hyperclamp0 %>% select(NR = TUEF_ID, AGE, BMI, PROCFETT)) %>%
```

```
  # Filtering out rows where 'gly.cat' is missing
  filter(!is.na(gly.cat))

# Creating a subset of data for the summary table
sumtab <- tabonedata %>% select(SEX, AGE, BMI, gly.cat) %>% ungroup()

# Creating a summary table using the 'tableone' package
t1 <- tableone::CreateTableOne(
  vars = names(tabonedata)[-1],  # Exclude 'NR' from the variables
  data = tabonedata,
  factorVars = c("SEX", "glycemia")  # Specifying categorical variables
)

# Printing the table with specific formatting for non-normal variables
print(t1, nonnoral = c("AGE", "BMI", "PROCFETT"))

# Generating an HTML version of the table
ht <- htmlTable(print(t1, nonnormal = c("AGE", "BMI", "PROCFETT")))

# Creating a file to save the HTML table
f1 <- file("tabl_HC.html", encoding = "UTF-8")

# Writing the HTML table to the file
cat(ht, file = f1)

# Closing the file connection
close(f1)

# This code block creates a well-structured summary table from the hyperclamp dataset,formats it using the 'tableone' package for both normal and non-normal variables, and exports it as an HTML file. The table includes demographic and clinical characteristics of the participants.
```

Computing and Displaying Correlations Stratified by Glycemic Group

```
# Prepare the data for stratified correlation analysis
cormtrx_strat_clamp <- clamp_cormtrx0 %>%
  # Group data by OGTT index, clamp index, and glycemia categories
  group_by(OGTT_index, clamp_index, glycemia) %>%
  # Filter out extreme outliers
  filter(
    abs(as.numeric(scale(OGTT_secretion))) < 5,
```

```
  abs(as.numeric(scale(clamp_secretion))) < 5
 ) %>%
 # Ensure complete cases for correlation analysis
 filter(complete.cases(OGTT_secretion, clamp_secretion)) %>%
 # Ensure there is more than one observation for each group
 filter(n() > 1) %>%
 # Summarize data to compute correlations and p-values for each group
 summarise(
  N = sum(complete.cases(OGTT_secretion, clamp_secretion)),
  cor_pearson = round(cor(x = OGTT_secretion, y = clamp_secretion, use =
"pairwise.complete.obs"), 3),
  cor_spearman = round(cor(x = OGTT_secretion, y = clamp_secretion, use =
"pairwise.complete.obs", method = "spearman"), 3),
  cor_pvalue = pformat(cor.test(x = OGTT_secretion, y = clamp_secretion, use =
"pairwise.complete.obs")$p.value),
  Sp_pvalue = pformat(cor.test(x = OGTT_secretion, y = clamp_secretion, use =
"pairwise.complete.obs", method = "spearman")$p.value)
 ) %>%
 # Arrange results by glycemia category and Spearman correlation
 arrange(glycemia, desc(cor_spearman))

# This code block calculates correlations between OGTT and clamp indices stratified by glycemic
categories.
# It ensures data quality by filtering out outliers and incomplete cases. The resulting data is
arranged by glycemia category and correlation strength for in-depth analysis.
```

Creating and Saving Tables for NGT Group Correlations in HC Data

```
# Enhance the correlation matrix with significance indicators for Spearman and Pearson
correlations
stern_HC <- cormtrx_strat_clamp %>%
 # Add significance indicators based on p-values for Spearman correlations
 mutate(Spearman_Cor = case_when(
  Sp_pvalue == "<0.05" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " *"),
  Sp_pvalue == "<0.01" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " **"),
  Sp_pvalue == "<0.001" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " ***"),
  TRUE ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " ")
 )) %>%
 # Add significance indicators for Pearson correlations
```

```
  mutate(Pearson_Cor = case_when(
    cor_pvalue == "<0.05" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " *"),
    cor_pvalue == "<0.01" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " **"),
    cor_pvalue == "<0.001" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " ***"),
    TRUE ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " ")
  )) %>%
  # Filter out specific clamp indices
  filter(clamp_index != "INS_ss_arg" & clamp_index != "CPEP_ss_arg")

# Prepare data for NGT group tables
cor_HC <- stern_HC %>%
  # Convert correlation values to numeric
  mutate(
    cor_spearman = as.numeric(cor_spearman),
    cor_pearson = as.numeric(cor_pearson),
    phase = case_when(
      clamp_index == "INS_ss_fp_glucose" | clamp_index == "CPEP_ss_fp_glucose" ~ "first",
      TRUE ~ "second"
    )
  ) %>%
  # Filter out specific clamp indices
  filter(clamp_index != "INS_ss_glp1" & clamp_index != "CPEP_ss_glp1")

# Create and save the table for the NGT group in the first-phase
tabl_NGT_HC <- cor_HC %>%
  filter(glycemia == "1_NGT" & phase == "first") %>%
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, values_from = c(Spearman_Cor, Pearson_Cor)) %>%
  arrange(row) %>%
  kbl() %>%
  kable_classic(full_width = F, html_font = "Cambria") %>%
  save_kable(file = "tabl_NGT_first_HC.html", self_contained = T)

# Create and save the table for the NGT group in the second-phase
tabl_NGT_HC <- cor_HC %>%
  filter(glycemia == "1_NGT" & phase == "second") %>%
```

```
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, values_from = c(Spearman_Cor, Pearson_Cor)) %>%
  arrange(row) %>%
  kbl() %>%
  kable_classic(full_width = F, html_font = "Cambria") %>%
  save_kable(file = "tabl_NGT_second_HC.html", self_contained = T)

# This code block creates and saves tables for the NGT group from hyperclamp data, highlighting
Spearman and Pearson correlations in both first and second-phases.
# The tables are saved as HTML files for easy viewing and documentation.
```

Creating and Saving Tables for Prediabetes Group Correlations in HC Data

```
# Create and save the table for the prediabetes group in the first-phase
tabl_prediab_HC <- cor_HC %>%
  filter(glycemia == "2_prediabetes" & phase == "first") %>%
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, values_from = c(Spearman_Cor, Pearson_Cor)) %>%
  arrange(row) %>%
  kbl() %>%
  kable_classic(full_width = F, html_font = "Cambria") %>%
  save_kable(file = "tabl_prediab_first_HC.html", self_contained = T)

# Create and save the table for the prediabetes group in the second-phase
tabl_prediab_HC <- cor_HC %>%
  filter(glycemia == "2_prediabetes" & phase == "second") %>%
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, values_from = c(Spearman_Cor, Pearson_Cor)) %>%
  arrange(row) %>%
  kbl() %>%
  kable_classic(full_width = F, html_font = "Cambria") %>%
  save_kable(file = "tabl_prediab_second_HC.html", self_contained = T)

# These code blocks create and save tables specifically for the prediabetes group from hyperclamp
```

data, highlighting Spearman and Pearson correlations in both the first and second-phases. The tables are saved as HTML files for documentation and presentation purposes.

Creating and Saving Tables for GLP1 Correlations in HC Data

```
# Prepare GLP1 data for table creation
GLP1_HC <- stern_HC %>%
  # Convert Spearman and Pearson correlation values to numeric
  mutate(
    cor_spearman = as.numeric(cor_spearman),
    cor_pearson = as.numeric(cor_pearson)
  ) %>%
  # Filter for GLP1-related clamp indices
  filter(clamp_index == "INS_ss_glp1" | clamp_index == "CPEP_ss_glp1")

# Create and save the table for the NGT group focusing on GLP1
tabl_GLP1_NGT_HC <- GLP1_HC %>%
  filter(glycemia == "1_NGT") %>%
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, values_from = c(Spearman_Cor, Pearson_Cor)) %>%
  arrange(row) %>%
  kbl() %>%
  kable_classic(full_width = F, html_font = "Cambria") %>%
  save_kable(file = "tabl_GLP1_NGT_HC.html", self_contained = T)

# Create and save the table for the Prediabetes group focusing on GLP1
tabl_GLP1_PreD_HC <- GLP1_HC %>%
  filter(glycemia == "2_prediabetes") %>%
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, values_from = c(Spearman_Cor, Pearson_Cor)) %>%
  arrange(row) %>%
  kbl() %>%
  kable_classic(full_width = F, html_font = "Cambria") %>%
  save_kable(file = "tabl_GLP1_rediab_HC.html", self_contained = T)

# These code blocks create and save tables highlighting the Spearman and Pearson correlations
```

for GLP1 in both the NGT and Prediabetes groups from the hyperclamp data. The tables are formatted and saved as HTML files for easy viewing and documentation.

Creating and Saving a Comprehensive Table for IVGTT Correlations

```
# Prepare the data for the Prediabetes group in the first-phase
kbl_NGT_IVGTT_first_0 <- stern_IVGTT %>%
  filter(glycemia == "2_prediabetes" & phase == "first") %>%
  select(row = OGTT_index, column = IVGTT_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, names_sep = "PREDIAB", values_from = c(Spearman_Cor,
Pearson_Cor)) %>%
  arrange(row)

# Prepare the data for the Diabetes group in the first-phase
kbl_NGT_IVGTT_first_1 <- stern_IVGTT %>%
  filter(glycemia == "3_diabetes" & phase == "first") %>%
  select(row = OGTT_index, column = IVGTT_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, names_sep = "_DM_", values_from = c(Spearman_Cor,
Pearson_Cor)) %>%
  arrange(row)

# Prepare and combine data for all glycemic groups in the first-phase
kbl_NGT_IVGTT_first <- stern_IVGTT %>%
  filter(glycemia == "1_NGT" & phase == "first") %>%
  select(row = OGTT_index, column = IVGTT_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, names_sep = "NGT", values_from = c(Spearman_Cor,
Pearson_Cor)) %>%
  left_join(kbl_NGT_IVGTT_first_0) %>%
  left_join(kbl_NGT_IVGTT_first_1) %>%
  arrange(row) %>%
  # Select specific columns for the final table
  select("row", contains("CorNGTINS_iAUC"), contains("NGTCP_iAUC_0"),
         contains("CorPREDIABINS_iAUC"), contains("PREDIABCP_iAUC_0"),
         contains("DM_INS_i"), contains("DM_CP_iAUC_0")) %>%
  filter(row != "AUC_I0_60_120_over_G0_60_120") %>%
  kbl() %>%
```

```
  kable_classic(full_width = F, html_font = "Cambria") %>%
  save_kable(file = "tabl_IVGTT.html", self_contained = T)

# This code block creates a detailed table for IVGTT correlations stratified by NGT, Prediabetes, and Diabetes groups in the first-phase. The table is formatted and saved as an HTML file for ease of access and documentation.
```

Creating and Saving a Comprehensive Table for HC Data

```
# Prepare data for NGT group in the second-phase
tabl_NGT_HC_s <- cor_HC %>%
  filter(glycemia == "1_NGT" & phase == "second") %>%
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, names_sep = "_NGT_Second_", values_from =
c(Spearman_Cor, Pearson_Cor))

# Prepare data for Prediabetes group in the first-phase
tabl_prediab_HC_f <- cor_HC %>%
  filter(glycemia == "2_prediabetes" & phase == "first") %>%
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, names_sep = "_Prediab_First_", values_from =
c(Spearman_Cor, Pearson_Cor))

# Prepare data for Prediabetes group in the second-phase
tabl_prediab_HC_s <- cor_HC %>%
  filter(glycemia == "2_prediabetes" & phase == "second") %>%
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, names_sep = "_Prediab_Second_", values_from =
c(Spearman_Cor, Pearson_Cor))

# Combine and prepare the final table for NGT group in the first-phase
tabl_NGT_HC <- cor_HC %>%
  filter(glycemia == "1_NGT" & phase == "first") %>%
  select(row = OGTT_index, column = clamp_index, Spearman_Cor, Pearson_Cor) %>%
  group_by(row) %>%
  pivot_wider(names_from = column, names_sep = "_NGT_First_", values_from = c(Spearman_Cor,
```

```
Pearson_Cor)) %>%
  left_join(tabl_prediab_HC_f) %>%
  left_join(tabl_NGT_HC_s) %>%
  left_join(tabl_prediab_HC_s) %>%
  select("row", contains("NGT_First_INS_ss_fp"), contains("NGT_First_CPEP_ss_fp"),
         contains("NGT_Second_INS_ss"), contains("NGT_Second_CPEP_ss"),
         contains("Prediab_First_INS_ss_fp"), contains("Prediab_First_CPEP_ss_fp"),
         contains("Prediab_Second_INS_ss"), contains("Prediab_Second_CPEP_ss")) %>%
  filter(row != "AUC_I0_60_120_over_G0_60_120") %>%
  arrange(row) %>%
  kbl() %>%
  kable_classic(full_width = F, html_font = "Cambria") %>%
  save_kable(file = "tabl_HC.html", self_contained = T)

# This code block creates and saves a detailed table combining NGT and Prediabetes groups'
correlations in both first and second-phases from the hyperclamp data. The table is formatted and
saved as an HTML file for ease of access and documentation.
```

Plotting Top 3 Correlations in HC Data for the Prediabetes Group

```
# Preparing data for the top 3 correlations plot
HC_top3 <- stern_HC %>%
  # Exclude specific clamp indices and non-significant correlations
  filter(!str_detect(clamp_index, "arg|glp1")) %>%
  filter(!cor_pvalue == "not" & !Sp_pvalue == "not") %>%
  select(OGTT_index, clamp_index, cor_spearman, glycemia, cor_pearson) %>%
  arrange(desc(cor_pearson), desc(cor_spearman)) %>%
  group_by(glycemia, clamp_index) %>%
  # Select the top 3 correlations for each group
  top_n(3) %>%
  ungroup() %>%
  # Classify phase based on clamp index
  mutate(phase = case_when(
    clamp_index == "INS_ss_fp_glucose" | clamp_index == "CPEP_ss_fp_glucose" ~ "First-phase",
    TRUE ~ "Second-phase"
  )) %>%
  # Filter for shared indices across glycemia groups
  mutate(
    shared = n_distinct(glycemia) == n_distinct(.$glycemia),
```

```
    cor_spearman = as.numeric(cor_spearman),
    cor_pearson = as.numeric(cor_pearson)
  ) %>%
  filter(shared == TRUE) %>%
  pivot_longer(col = c(cor_pearson, cor_spearman)) %>%
  # Focus on the second-phase for Prediabetes group
  filter(phase != "First-phase" & glycemia == "2_prediabetes")

# Create the plot using ggplot2
ggplot(HC_top3, aes(x = reorder(OGTT_index, value), y = value, fill = OGTT_index, group = name,
alpha = name)) +
  geom_bar(stat = "identity", position = position_dodge()) +
  # Facet the plot for different categories
  facet_wrap(vars(glycemia, phase, clamp_index), scales = "free", ncol = 4, labeller =
labeller(clamp_index = column1, glycemia = glycemia1)) +
  theme_nice() +
  scale_alpha_manual("Correlation coefficients", values = c(1, 0.5), labels = c("Pearson",
"Spearman")) +
  scale_x_discrete(labels = indeces) +
  # Customize theme for readability
  theme(
    legend.text = element_text(size = 17),
    legend.title = element_text(size = 17),
    text = element_text(size = 17),
    title = element_text(size = 15),
    strip.text = element_text(size = 15),
    axis.text = element_text(size = 11),
    axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)
  ) +
  ylab("") +
  scale_y_continuous("", limits = c(0, 1)) +
  xlab("") +
  scale_fill_brewer("Indices", labels = indeces, palette = "Set1")

# Save the plot as a PNG file with specified dimensions
ggsave("HC_3top.png", width = 23, height = 15, units = "cm")


# This code block generates a detailed bar plot showing the top three correlations in hyperclamp
```

data for the Prediabetes group, focusing on the second-phase. The plot is saved as a high-resolution image for presentation or documentation purposes.

Plotting Top Correlations in HC Data Covering Both Phases

```
# Enhance the correlation matrix with significance indicators for correlations
stern_HC_b <- clamp_cormtrx %>%
  # Add significance indicators based on p-values for Spearman correlations
  mutate(Spearman_Cor = case_when(
    Sp_pvalue == "<0.05" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " *"),
    Sp_pvalue == "<0.01" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " **"),
    Sp_pvalue == "<0.001" ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " ***"),
    TRUE ~ paste0(str_extract(cor_spearman, "^\\d.+|^-\\d.+"), " ")
  )) %>%
  # Add significance indicators for Pearson correlations
  mutate(Pearson_Cor = case_when(
    cor_pvalue == "<0.05" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " *"),
    cor_pvalue == "<0.01" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " **"),
    cor_pvalue == "<0.001" ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " ***"),
    TRUE ~ paste0(str_extract(cor_pearson, "^\\d.+|^-\\d.+"), " ")
  )) %>%
  # Filter out specific clamp indices
  filter(clamp_index != "INS_ss_arg" & clamp_index != "CPEP_ss_arg")

# Prepare the data for the top correlations plot
NDv_HC <- stern_HC_b %>%
  filter(!cor_pvalue == "not" & !Sp_pvalue == "not") %>%
  filter(clamp_index != "INS_ss_glp1" & clamp_index != "CPEP_ss_glp1") %>%
  arrange(desc(cor_pearson), desc(cor_spearman)) %>%
  group_by(clamp_index) %>%
  # Select the top 7 correlations for each clamp index
  top_n(7) %>%
  ungroup() %>%
  group_by(OGTT_index) %>%
  # Classify phase based on clamp index
  mutate(phase = case_when(
    clamp_index == "INS_ss_fp_glucose" | clamp_index == "CPEP_ss_fp_glucose" ~ "First-phase",
    TRUE ~ "Second-phase"
  )) %>%
```

```
  # Filter for shared indices across groups
  mutate(
    shared = n_distinct(clamp_index) == n_distinct(.$clamp_index),
    cor_spearman = as.numeric(cor_spearman),
    cor_pearson = as.numeric(cor_pearson)
  ) %>%
  filter(shared == TRUE) %>%
  pivot_longer(col = c(cor_pearson, cor_spearman))

# Create the plot using ggplot2
ggplot(NDv_HC, aes(x = reorder(OGTT_index, -value), y = value, fill = OGTT_index, group = name,
alpha = name)) +
  geom_bar(stat = "identity", position = position_dodge()) +
  # Facet the plot for different phases and clamp indices
  facet_wrap(vars(phase, clamp_index), scales = "free_x", ncol = 2, labeller = labeller(clamp_index =
column1, glycemia = glycemia1)) +
  theme_nice() +
  scale_alpha_manual("Correlation coefficients", values = c(1, 0.5), labels = c("Pearson",
"Spearman")) +
  scale_fill_discrete("Indices", labels = indeces) +
  scale_x_discrete(labels = indeces) +
  # Customize theme for readability
  theme(
    legend.text = element_text(size = 17),
    legend.title = element_text(size = 17),
    text = element_text(size = 17),
    title = element_text(size = 17),
    strip.text = element_text(size = 17),
    axis.text = element_text(size = 15),
    axis.text.x = element_text(angle = 90, vjust = 0.5, hjust = 1)
  ) +
  ylab("") +
  scale_y_continuous("", limits = c(0, 1)) +
  xlab("")

# Save the plot as a PNG file with specified dimensions
ggsave("HC_top_both.png", width = 20, height = 25, units = "cm")
# This code block generates a detailed bar plot showing the top correlations in hyperclamp data for
```

both first and second-phases. The plot is saved as a high-resolution image for presentation or documentation purposes.

Creating and Saving a Plot for Top Correlations from Both HC and IVGTT Data

```
# Prepare data from HC for plotting
NDv_HC_B <- stern_HC_b %>%
  filter(!cor_pvalue == "not" & !Sp_pvalue == "not" & !str_detect(clamp_index, "arg|glp")) %>%
  select(row = OGTT_index, column = clamp_index, cor_spearman, cor_pearson)
# Prepare data from IVGTT for plotting
NDv_IVGTT_B <- stern_IVGTT_b %>%
  select(row = OGTT_index, column = IVGTT_index, cor_spearman, cor_pearson)
# Combine and arrange data from both HC and IVGTT
Both <- rbind(NDv_IVGTT_B, NDv_HC_B) %>%
  arrange(desc(cor_spearman), desc(cor_pearson)) %>%
  group_by(column) %>%
  # Select the top 8 correlations for each group
  top_n(8) %>%
  group_by(row) %>%
  # Determine if there are shared columns across groups
  mutate(
    shared = n_distinct(column) == n_distinct(.$column),
    phase = case_when(
      column == "CP_iAUC_10_60" | column == "INS_iAUC_10_60" | column == "CPEP_ss_glucose" |
column == "INS_ss_glucose" ~ "Second-phase",
      TRUE ~ "First-phase"
    )
  ) %>%
  filter(shared == TRUE) %>%
  pivot_longer(col = c(cor_pearson, cor_spearman)) %>%
  filter(row != "BIGTT_AIR_0_30_120") %>%
  mutate(column = replace(column, column == "INS_iAUC_0_10", "CINS_iAUC_0_10"))

# Create the plot using ggplot2
ggplot(Both, aes(x = reorder(row, value), y = value, fill = row, group = name, alpha = name)) +
  geom_bar(stat = "identity", position = position_dodge()) +
  # Facet the plot for different columns
  facet_wrap(vars(column), scales = "free", ncol = 2, labeller = labeller(column = column1)) +
```

```
  theme_nice() +
  scale_alpha_manual("Correlation coefficients", values = c(1, 0.5), labels = c("Pearson",
"Spearman")) +
  scale_fill_viridis_d("Indices", labels = indeces) +
  scale_x_discrete(labels = indeces) +
  # Customize theme elements for readability and aesthetics
  theme(
    legend.text = element_text(size = 25),
    legend.title = element_text(size = 25),
    text = element_text(size = 20),
    title = element_text(size = 20),
    strip.text = element_text(size = 21),
    axis.title.x = element_blank(),
    axis.text.x = element_blank(),
    axis.ticks.x = element_blank()
  ) +
  ylab("") +
  xlab("")

# Save the plot as a high-resolution PDF file with specified dimensions
ggsave("Figure 1.pdf", width = 40, height = 45, units = "cm", dpi = 500)
# This code block generates a detailed bar plot showing the top correlations from both HC and
IVGTT data. The plot is saved as a high-resolution PDF for documentation and presentation
purposes.
```

Creating and Saving a Plot for Top GLP1 Correlations in HC Data

```
# Prepare GLP1-related data from Hyperclamp for plotting
NDv_GPL1_0 <- stern_HC_b %>%
  filter(!cor_pvalue == "not" & !Sp_pvalue == "not" & str_detect(clamp_index, "glp")) %>%
  select(row = OGTT_index, column = clamp_index, cor_spearman, cor_pearson)
# Arrange and select the top correlations for GLP1
NDv_GPL1 <- NDv_GPL1_0 %>%
  arrange(desc(cor_spearman), desc(cor_pearson)) %>%
  group_by(column) %>%
  # Select the top 8 correlations for each group
  top_n(8) %>%
  filter(row != "BIGTT_AIR_0_60_120") %>%
  group_by(row) %>%
```

```
  # Determine if there are shared columns across groups
  mutate(shared = n_distinct(column) == n_distinct(.$column)) %>%
  filter(shared == TRUE) %>%
  pivot_longer(col = c(cor_pearson, cor_spearman))

# Create the plot using ggplot2
ggplot(NDv_GPL1, aes(x = reorder(row, -value), y = value, fill = row, group = name, alpha = name)) +
  geom_bar(stat = "identity", position = position_dodge()) +
  # Facet the plot for different GLP1-related columns
  facet_wrap(vars(column), scales = "free", ncol = 2, labeller = labeller(column = column1, glycemia
= glycemia1)) +
  theme_nice() +
  scale_alpha_manual("Correlation coefficients", values = c(1, 0.5), labels = c("Pearson",
"Spearman")) +
  scale_fill_viridis_d("Indices", labels = indeces, option = "C") +
  scale_x_discrete(labels = indeces) +
  # Customize theme elements for readability and aesthetics
  theme(
    legend.text = element_text(size = 25),
    legend.title = element_text(size = 25),
    text = element_text(size = 20),
    title = element_text(size = 20),
    strip.text = element_text(size = 21),
    axis.title.x = element_blank(),
    axis.text.x = element_blank(),
    axis.ticks.x = element_blank()
  ) +
  ylab("") +
  scale_y_continuous("", limits = c(0, 1)) +
  xlab("")

# Save the plot as a high-resolution PDF file with specified dimensions
ggsave("Figure 2.pdf", width = 30, height = 25, units = "cm", dpi = 500)

# This code block generates a detailed bar plot showing the top correlations related to GLP1 in
hyperclamp data. The plot is saved as a high-resolution PDF for documentation and presentation
purposes.
```