Diplomarbeit

Effects of prolonged fasting on glucose metabolism and hormone regulation in healthy, obese, and people with type 2 diabetes

eingereicht von

Matthias Zanker

zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an der

Abteilung für Endokrinologie und Diabetologie

unter der Anleitung von

Herr Assoz. Prof. Priv.-Doz. Dr.med. univ. Harald Sourij

Graz, 27.08.2020

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 27.08.2020 *Matthias Zanker eh*

Vorwort

Das Thema „Fasten“ gewinnt vor allem in der westlich geprägten Welt zunehmend an Bedeutung. Ein dauerhaftes Nahrungsüberangebot in diesen Ländern führt beim Großteil der Bevölkerung zu einer unverhältnismäßigen Nahrungsaufnahme. Die Folge ist eine steigende Anzahl an Menschen mit Übergewicht und Volkskrankheiten wie Typ-2-Diabetes oder kardiovaskulären Erkrankungen. Jedoch hat sich in den letzten Jahren ein Trend in Richtung eines „gesunden Lifestyles“ entwickelt. Um jenen gesunden Lebensstil auszuüben wird man vor eine unübersichtliche Auswahl an Diäten gestellt, sei es die ketogene, Paleo- oder Low Carb-Diät. Ebenso hat man die Möglichkeit ohne radikale Umstellung der Nahrungsbestandteile eine gesündere Lebensweise zu erzielen, nämlich durch simples Fasten. Hier stellt intermittierendes Fasten eine der populärsten Methoden dar, bei der man über eine Dauer von 16 bis 24 Stunden jegliche Kalorienzufuhr unterbindet. Es gibt wissenschaftlich fundierte Hinweise darauf, dass diese Form des Fastens eine reversible Wirkung auf Typ 2 Diabetes und Übergewicht hat. Inwiefern sich der Metabolismus bei Menschen mit Diabetes und Übergewichtigen verändert, wenn man die Dauer des Nahrungsentzuges verlängert ist allerdings noch nicht bekannt und wird in dieser Studie untersucht. Welche Diät oder Methode des Fastens man wählt wird vermutlich eine individuelle Entscheidung bleiben, aber anhand von wissenschaftlichen Daten ist es möglich deren Vor- und Nachteile besser zu beleuchten.

Danksagungen

Ich möchte mich in erster Linie bei meinem professionellen und mentoriellen Erstbetreuer Assoz. Prof. Priv.-Doz. Dr.med. univ. Harald Sourij bedanken, der mir die Möglichkeit gegeben hat an einer so umfassenden und äußerst spannenden Studie aktiv teilzuhaben. Da ich von Anfang an Teil der PROLOG-Studie war und meine Mitarbeit immer sehr geschätzt wurde bereiteten mir die Studienvisiten besonders große Freude.

An dieser Stelle geht auch ein großes Dankeschön an meine großartigen Mitbetreuer Dr. Norbert Tripolt und Peter Pferschy, MSc mit jenen ich am Studienzentrum der klinischen Abteilung für Endokrinologie und Diabetologie Graz den Großteil der Visiten durchführen durfte. Ebenso danke ich dem gesamten Team der klinischen Abteilung für Endokrinologie und Diabetologie für die frühmorgendliche Verfügbarkeit zur Glukoseinfusion.

Zu guter Letzt bedanke ich mich bei meinen Eltern, welche mir das Medizin-Studium ermöglicht und mich fortwährend unterstützt haben, auch wenn das Lernangebot meine Nachfrage oft mehrfach gedeckt hat.

Zusammenfassung

In dieser Studie wurde der Effekt von 12h und 36h Fasten auf den Blutzuckerspiegel und die postprandiale Glukoseverwertung in Normalgewichtigen, Adipösen und Typ-2-Diabetikern untersucht. Alle Teilnehmer der drei Gruppen unterliefen einer zweiteiligen prospektiven Fasten-Interventionsstudie, wobei sie bei der ersten Visite 12 Stunden und bei er zweiten Visite 36 Stunden fasteten. Anhand eines oralen Glukose Toleranztest (OGTT) konnten Rückschlüsse auf den Glukosemetabolismus, die glukoregulatorischen Hormone und die Insulinsekretion gezogen werden.

Es wurden 60 ProbandInnen (Alter: 43±16 Jahre, 62% weiblich) in die Studie inkludiert. Davon waren 20 normalgewichtig (11m, 9w, Alter: 32±10 Jahre, BMI: 22.6±1.7 kg/m²), 20 adipös (11m, 9w, Alter: 37±10 Jahre, BMI: 34.6±4.8 kg/m²) und 20 Menschen mit Typ-2-Diabetes (15m, 5w, Alter: 60±9Jahre, BMI: 31.0±7.1 kg/m²). Nach 36 h fasten im Vergleich zu 12h traten folgende Ergebnisse auf: Nüchternglukose- und Insulinplasmaspiegel waren nach 36 Stunden im Vergleich zu 12 Stunden fasten in allen drei Versuchsgruppen signifikant reduziert. Ebenso verbesserte sich nach 36 Stunden Fasten die Insulinsensitivität in den drei Kohorten deutlich, welche anhand von QUICKI und HOMA-IR bestimmt wurde (**HOMA-IR** 12h vs. 36h: Normalgewichtige 0.86(0.75 – 1.41) vs. 0.43 (0.27 – 0.67), p=0.001; Adipöse 2.52 (1.72 – 4.27) vs. 1.37 (0.90 – 3.07), p=0.001; T2DM 4.26±2.06 vs. 3.17±2.17, p=0.012; **QUICKI** 12h vs. 36h: Normalgewichtige 0.39 (0.37 – 0.40) vs. 0.45 (0.41 – 0.49), p=0.000; Adipöse 0.33 (0.31 – 0.35) vs. 0.36 (0.32 – 0.39), p=0.021; T2DM 0.31 (.30 – 0.33) vs. 0.32 (0.31 – 0.37), p=0.001). Postprandial kam es ausschließlich bei den Normalgewichtigen Probanden nach der 36-stündigen Nahrungskarenz zu einem signifikanten Anstieg des Plasmaglukosespiegels 120min nach Beginn des OGTT (PG 120min: 12h: 79.4±18.4 vs 36h: 108.7±31.0 mg/dL, p=0.001) und der Glucose AUC (Glucose AUC: 12h: 17070±3128 vs. 21627±4002 mg min/dL, p=0.000).

In Conclusio führt einmaliges prolongiertes Fasten zu einem signifikanten postprandialen Blutzuckerstieg bei Gesunden, zu einer numerischen jedoch nicht signifikanten Erhöhung in Adipösen und bei Menschen mit Typ-2-Diabetikes ist kein Unterschied erkennbar.

Abstract

In this study we investigated the effect of midterm-fasting (36h) compared to overnight fasting (12h) on plasma glucose levels and postprandial glucose disposal in healthy, obese, and people with type 2 diabetes. During a two-part prospective fasting intervention-study, all participants fasted for 12 hours for the first and 36 hours for the second visit. To evaluate the changes of glucose metabolism, glucoregulatory hormones and insulin response we performed an oral glucose tolerance test (OGTT) following 12 and 36 hours of fasting.

In total we included 60 participants in this study (f, m, age: 23 to 74 years). From those 60 subjects 20 were healthy (11m, 9f, age: 32±10 years, BMI: 22.6±1.7 kg/m²), 20 obese (11m, 9f, age: 37±10 years, BMI: 34.6±4.8 kg/m²) and 20 were people with type 2 diabetes (15m, 5f, age: 60±23.9 years, BMI: 31.0±7.1 kg/m²). Following results were obtained after 12 and 36 hours of fasting: Fasting glucose- and insulin values were significantly reduced in all three study groups after 36 hours of fasting. Insulin sensitivity, determined by HOMA-IR and QUICKI, was remarkably increased following the prolonged fasting of 36 hours (HOMA-IR 12h vs. 36h: Healthy 0.86(0.75 – 1.41) vs. 0.43 (0.27 – 0.67), p=0.001; Obese 2.52 (1.72 – 4.27) vs. 1.37 (0.90 – 3.07), p=0.001; T2DM 4.26±2.06 vs. 3.17±2.17, p=0.012; QUICKI 12h vs. 36h: Healthy 0.39 (0.37 – 0.40) vs. 0.45 (0.41 – 0.49), p=0.000; Obese 0.33 (0.31 – 0.35) vs. 0.36 (0.32 – 0.39), p=0.021; T2DM 0.31 (.30 – 0.33) vs. 0.32 (0.31 – 0.37), p=0.001). Postprandial changes were only observed in healthy subjects as the plasma glucose levels 120 minutes post- OGTT and the glucose AUC notably increased after having fasted for 36 hours. (PG 120min 12h vs. 36h: 79.4±18.4 vs. 108.7±31.0 mg/dL, p=0.001; Glucose AUC 12h vs. 36h: 17070±3128 vs. 21627±4002 mg min/dL, p=0.000).

In conclusion a one-time fasting period of 36 hours causes a significant postprandial glucose increase in healthy, a similar however non-significant effect in obese and no difference in type 2 diabetes.

Inhaltsverzeichnis

Inhalt

Abbildungsverzeichnis

Tabellenverzeichnis

1 Introduction

1.1 Food restriction/Fasting in healthy, obese, and type 2 diabetes

Alternate day fasting (ADF) is becoming an increasingly popular form of diet. It defines an eating regime including a complete energy restriction over a certain period following ad libitum feeding interval. Different studies support the healthy benefits of ADF such as lowering the body weight, reducing glucose and insulin levels and increasing resistance to endotoxic stress (1). In one of these studies a similar approach to our investigations was chosen to enlighten the effects of midterm fasting in non-obese healthy adults. Heilbronn et. al showed that fasting insulin levels after a 36-hour fasting period was significantly lower than after fasting of only 12 hours.(1) The fasting glucose however did not vary between the 36- and 12-hour food absence. A drop in fasting insulin and in contrast to Heilbronn et al. also a decrease in fasting glucose was shown in a 1-day fasting intervention study (28 hours fasting) including 30 healthy adults.(2) A slightly shorter period of complete energy restriction (20 hours) in non-obese healthy adults had the effect of lowering fasting glucose however did not show any significant effects on the fasting insulin levels different to the other studies (1), (2). Little evidence is available for alternate day fasting in obese adults and in people with type 2 diabetes. One study by Corley et. al evaluated the risk of hypoglycemic events during 5:2 fasting for 12 weeks in Type 2 Diabetes Mellitus patients. The results demonstrated T2DM patients while being on blood glucose lowering medication have an elevated risk of hypoglycemia during fasting days. Furthermore the study presented an approach to reduction of medication due to the lack of glucose intake on fasting days and the consequent weight loss. (3) Furmli et al has proven that is possible to reduce antidiabetic medication as two of three insulin dependent patients with type 2 diabetes were able to discontinue their insulin treatment and all three could get rid of their oral antidiabetic medication after following an ADF regime for 7-11 months.(4) These results pose the question of an improved insulin sensitivity and/or glucose disposal after fasting in people with type 2 diabetes, however there is contrary evidence stating that fasting in T2DM patients leads to no significant increase in insulin sensitivity. (5) The same study however allows subjects an intake of maximum 300 kcal per day, which

defines calorie restriction (CR) rather than ADF where no caloric intake is allowed during fasting periods.

Alternate day modified fasting also known as calorie restriction is another form of fasting which allows an energy intake well below the normal daily consumption. Gabel et al examined the differences of alternate day fasting and calorie restriction. ADF in comparison to CR lead to a stronger decrease of fasting insulin and to a greater reduction of insulin resistance (6). The effect of CR on obese subjects were examined by many studies presenting very incoherent findings. On the one side Hoddy et al's study including obese subjects performing an 8-week long weight-loss diet with alternate day modified fasting allowing them an energy intake of 25% of their daily needs, demonstrated a reduction in glucose and insulin levels at the end of the trial (7). On the other hand a comparable study design created and implemented by Bhutani et al (12 weeks intervention of CR with 25% of energy needs) presented no significant changes in these glucoregulatory markers(8).

1.2 Fasting and consecutive food intake:

The stimulation of postprandial insulin production mainly depends upon glucose. Amino acids and free fatty acids also contribute to the stimulation of insulin secretion. Beta cells sense the concentration of these three micronutrients, which are consequently metabolized by the beta cells mitochondria and additionally adapt the need of insulin.(9) Neuronal and endocrine responses influence the insulin secretion as well,(10) however in this study we mainly focus on the glucose stimulated insulin secretion. Total energy restriction generates a scarcity of glucose, FFA and amino acids creating the need of an alternative energy source besides glucose. A metabolic switch from glycolysis to FFA-oxidation to cover the energy demands of peripheral tissue has been described to occur after 24 hours of fasting when hepatic glycogen stores are emptied. (11) Insulin is suppressed during fasting to efficiently activate lipolysis and make FFAs sufficiently accessible.(12) This mechanism serves to protect glucose to be predominantly used by the brain. In a postprandial state following prolonged starvation periods, glucose disposal impairments have already been described in healthy subjects. In this study we try to investigate the effects of prolonged fasting in healthy, obese, and subjects with type 2 diabetes.

2 Material and Methods

Participants: In total a study population of 60 participants aged 23 to 74 years were recruited from the Medical University of Graz. Of those 57 participants 20 were healthy, 20 obese and 20 people with type 2 diabetes.

We filtered the participants according to our inclusion and exclusion criteria as follows:

Inclusion Criteria Cohort I (Healthy, non-obese subjects)

- ➔ Age >18 years
- ➔ Body mass index in the range of 20.0 – < 27.0 kg/m2,
- ➔ Fasting plasma glucose <110mg/dL (without medication)

Exclusion criteria cohort I

- ➔ Presence of any metabolic disease
- ➔ Intake of any glucose lowering, lipid lowering or blood pressure lowering medication

Inclusion Criteria Cohort II (obese subjects)

- ➔ Age >18 years
- ➔ Body mass index > 30.0 kg/m2
- ➔ Fasting plasma glucose <110mg/dL (without medication)

Exclusion criteria cohort II

- ➔ Intake of any glucose lowering medication

Inclusion Criteria Cohort III (Subjects with Type 2 Diabetes)

- ➔ Age >18 years
- ➔ established diabetes mellitus type 2 on either diet or a monotherapy or combination of metformin, DPP-4-inhibitors or sulfonylurea

Exclusion Criteria for all participants

- ➔ No history of cardiovascular disease
- ➔ No acute or chronic inflammatory disorder
- ➔ No heavy drinking (more than 15 drinks/week)
- ➔ No dietary restrictions (e.g. vegetarianism and vegan)
- ➔ Insulin treatment

- ➔ Known Malignancy
- ➔ Women who are pregnant, breast-feeding or trying to become pregnant
- ➔ History of any chronic disease process that could interfere with interpretation of study results
- ➔ Therapy with antidepressants within past 6 months
- ➔ Therapy with glucocorticoids

The study was approved by the Ethics Committee of the Medical University of Graz (30-238 ex 17/18), Austria and was conducted according to the Declaration of Helsinki, GCP-ICH and according to the protocol and the requirements of the concerned regulatory authorities. All study participants signed an Informed Consent beforehand.

General protocol: All participants had to complete two visits over the course of 7+- 2 days. On day 1 the first visit was performed after the subjects fasted 12hours (overnight fasting) and ideally on day 7 (within the range of +- 2 days) the second visit followed the prolonged fasting period of 36 hours. The procedure after both fasting periods was performed according to the study protocol with the subjects undergoing a bioimpedance analysis and receiving an oral glucose tolerance test.

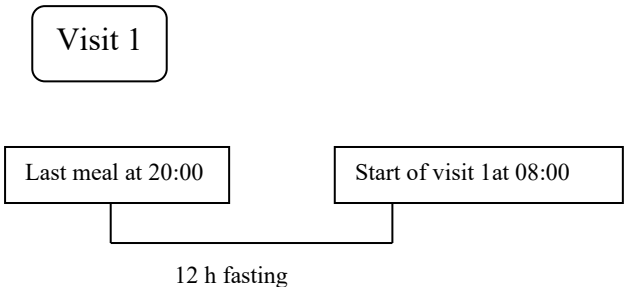


Figure 1 Graphic representation of the workflow of visit 1.

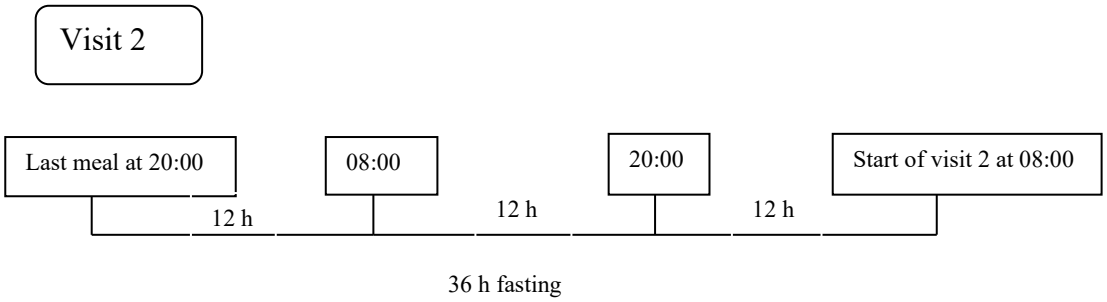


Figure 4 Graphic representation of the workflow of visit 2.

Oral glucose tolerance test:

In order to measure hepatic and peripheral insulin sensitivity we used several insulin sensitivity indices generated by multiple blood samples drawn after administration of the oral glucose tolerance test. The OGTT for the first visit was performed after an overnight fast (12h) whereas for the second visit subjects had to stay in a fasting state for 36 continuous hours. We aimed towards a 100% calorie restriction during the fasting periods only allowing the intake of water, unsweetened tea, and coffee (no milk added). The baseline blood sample was taken at -5min, followed by the ingestion of 75g glucose (Glucoral 75 citron, Germania Pharmazeutika, Vienna) for which the subjects were given 2-4 minutes to set the timepoint of 0 min. During the OGTT, blood was drawn to measure Insulin, C-peptide and plasma glucose at the timepoints 15, 30, 60, 120 and 180min. An intravenous line for blood drawing was established and repeatedly flushed with 0,09 % saline solution to avoid the formation of blood clots. At each of the five timepoints we used one fluoride oxalate tube (1mL) to measure plasma glucose and one serum tube to analyze insulin and c-peptide levels.

The results of the OGTT were integrated into following equations to determine the **fasting insulin sensitivity and postprandial insulin sensitivity**:

Fasting insulin sensitivity:

HOMA-IR (Homeostasis Model Assessment for Insulin Resistance):

$$HOMA - IR = \frac{FPG\left(\frac{mmol}{l}\right) * FSI\left(\frac{U}{l}\right)}{22.5}$$

and

Quantitative Insulin sensitivity Check Index (QUICKI):

$$QUICKI = \frac{1}{\log(insulin0) + \log(glucose0)}$$

Postprandial insulin sensitivity:

Insulin sensitivity index (ISI) proposed by Stumvoll et al.:

$$ISI = 0.222 - 0.00333 * BMI - 0.0000779 * Ins120 - 0.000422 * age$$

Beta cell function was calculated with:

Homeostatic model assessment for beta cell function (HOMA-β) to evaluate *beta cell function in fasting state*:

$$HOMA - \beta = \frac{20 * insulin0}{Glucos0 - 3.5}$$

Stumvoll Index (1st and 2nd phase) and $OGTT_{ratio}$ applied for estimating *postprandial insulin response and glucose disposal*:

$$1st\ phase = 1283 + 1.829 * Ins30 - 138.7 * Glc30 + 3.772 * Ins0$$

$$2nd\ phase = 286 + 0.416 * Ins30 - 25.94 * Glc30 + 0.926 * Ins0$$

The change of insulin and glucose plasma levels 30 minutes after OGTT initiation was measured with the OGTT ratio formula: $OGTT_{ratio} = \frac{\Delta insulin30}{\Delta glucose30}$

Laboratory measurements

Measurements of Insulin and c-peptide were performed by chemiluminescence on an ADVIA Centaur system (Siemens, Healthcare Diagnostics, Eschborn, Germany). Thyroid hormones such as triiodothyronine, thyroxine, thyrotropin, and stress hormone cortisol were analyzed by Siemens ADVIA Centaur, Eschborn, Germany. Leptin, a hormone signaling hunger was centrifuged and kept at -80°C until analysis. (*Levels of free fatty acids in the blood plasma were obtained by/via an in vitro enzymatic colorimetric method assay by Wako chemicals Neuss, Germany on an Olympus AU640 (Olympus Diagnostica Hamburg, Germany)*). Routine parameters were measured with a cobas® analyzer (Roche Diagnostics, Mannheim, Germany). Using ELISA-Kits (Mercodia AB, Uppsala, Sweden) we analyzed glucagon. Proinsulin-levels were tested by using a quantitative Immunoassay (MLT Research Limited, Cardiff, UK).

Blood sampling and storage

Within the two study visits circa 100mL of blood was drawn from each subject to measure the above-mentioned laboratory parameters. After analyzing the blood, a surplus of serum and plasma was stored at -80°C in the Biobank of the Medical University of Graz in order to perform additional testing of plasma amino acids and lipid metabolism. Subjects agreed to this routine by signing an informed consent prior to the first visit.

Physical examination

The subject's general appearance, cardiovascular system, respiratory system, skin, musculoskeletal system, central and nervous system were examined at the first visit.

Vital Signs

Subjects Systolic and diastolic blood pressure were recorded at Visit 1 and Visit 2. When arriving at the trial site participants were asked to sit comfortably with uncrossed legs, back and arms supported for at least five minutes for an unbiased blood pressure measurement. No talking is allowed during the examination. Parallel to the RR-measurement the pulse (bpm) was being evaluated as well for both visits.

Body composition (Bioelectrical impedance analysis – BIA)

Bioelectrical impedance analysis is a very reliable tool to evaluate a person's body composition. With the help of different electrical frequencies which are vital to determine between more conductive tissue (muscle) and less conductive tissue (fat) the fat free mass and fat mass can be estimated by the Biacorpus RX 40000. A total of eight electrodes, two for each extremity, are placed on the subject's hands and feet followed by an automatic 20 second measuring interval. Before this process is initiated by pressing the "new measurement" button in the Biacorpus software user interface (installed on a PC/Laptop) the examiner must enter the subject's age, weight, and height. Because the PC is connected to the Biacorpus device via USB interface, data is automatically transferred into the user's software ready and compatible for statistical analyzing.

Data analysis

Primary outcome questioning the difference in OGTT after a 12-hour vs. a 36-hour fasting period was calculated using Student's t-tests for paired data and Wilcoxon signed rank test for unpaired, respectively.

Sample size estimation

We estimated that a sample size for each cohort of n=20 participants will give an effect size of about 0.7 detectable with a power of 80%. Three same-sized cohorts added up to a total of 60 participants.

3 Results

In total *n* = 60 Participants were recruited for this study. We had three groups: healthy (*n* = 20), obese (*n* = 20) and people with type 2 diabetes (*n* = 20). Baseline characteristics for the three study groups are as follow:

- Healthy: mean Age = 32+-10 years, BMI, mean= 22.6±1.7kg/m² ,11 males, 9 females.
- Obese: mean Age = 37+-10 years, BMI, mean = 34.6±4.8 kg/m², 11 males, 9 females.
- T2DM: mean Age = 60+-9 years, BMI, mean 31±7.1 kg/m², 15 males, 5 females.

3.1 Fasting Glucose Metabolism 12 vs 36 hours:

Fasting levels of glucose, insulin, c-peptide, and proinsulin after the 12h and 36h long energy restriction are shown on Table 1.

Fasting glucose 12 vs 36 h: The fasting glucose was significantly lower after 36h of fasting than after 12h in all three study populations, non-obese (p= 0.003), obese (p= 0.002) and Type 2 DM (p= 0.017). After the prolonged total ER period of 36 hours healthy subjects exhibited a fasting baseline plasma glucose value of 73.4 ± 10.6 vs 80.3 ± 7.3 mg/dL (p= 0.003) after 12h of fasting. Obese subject's prolonged fasting (36h) PG of 87.5 ±12.6 was remarkably lower than after an overnight fast (12h) measuring 93.7 ± 9.9 mg/dL (p= 0.002). The group including people with type 2 diabetes showed a notable drop in baseline PG from 149.9 ± 36.9 to 135.7 ± 26.3 mg/dL (p= 0.017) having fasted 12h and 36h, respectively.

Fasting insulin 12 vs 36h: Fasting insulin levels were significantly lower in all three groups comparing 12 vs 36 hours of fasting showing a drop in healthy subjects from 4.3 (4.0-7.1) to 2.5 (1.8-4.1) mU/L (p= 0.002), in obese from 11.4 (6.7-15.9) to 7.2 (5.0-14.5) mU/L (p= 0.020) and in people with type 2 diabetes from 11.2 ± 4.4 to 9.2 ± 5.8 mU/L (p= 0.007).

Fasting c-peptide 12 vs 36h: C-peptide fasting levels, one of the two cleavage products of proinsulin exhibited similar reductions in all three study groups. We observed significant decrease in c-peptide levels after 36h vs 12h of fasting in healthy (0.94(0.83-1.13) vs. 0.61(0.43-0.82) ng/mL, p= 0.001), in obese (2.00(1.55-2.46) vs 1.65(0.9-2.24) ng/mL, p= 0.036) and subjects with type 2 diabetes (2.31 ± 0.75 vs 1.94+-1.02 ng/mL, p= 0.002).

Since we detected a significant decrease in baseline insulin after prolonged fasting in all three cohorts we anticipated a similar change in c-peptide as both are split products from proinsulin with c-peptide having a longer half-life (20-30min) compared to insulin (3-5min). (13)

Fasting proinsulin 12 vs 36h: Fasting Proinsulin was only reduced significantly in the DM2 group after the 36h fast.

Fasting Insulin sensitivity indices

HOMA-IR 12vs 36h: HOMA-IR, QUICKI are useful tools to measure hepatic rather than peripheral insulin resistance in a fasting state.(14) Insulin resistance was calculated with HOMA-IR. (15)(16) Following the 36 hours of total calory restriction all of the three study groups showed a significant decrease in insulin resistance in comparison to the 12 hours of fasting. Healthy subjects showed HOMA-IR values of 0.86 (0.75-1.41) after 12h and 0.43 (0.27-0.67) after 36 hours with a p-value of 0.001. A significant drop (p= 0.001) in HOMA-IR from 2.52 (1.72 - 4.27) after 12h of fasting to 1.37 (0.90 - 3.07) following 36 hours of fasting was seen in obese individuals. Participants with type 2 diabetes revealed a remarkable decrease (p= 0.001) in HOMA-IR after the prolonged fasting with 3.17 ± 2.17 compared to an overnight fasting value of 4.26 ± 2.06.

QUICKI 12 vs 36h: All three groups displayed a significant increase in QUICKI when having fasted for 36 hours compared to 12 hours of fasting. The healthy group displayed a significant increase in QUICKI from 0.39 (0.37 – 0.40) to 0.45 (0.41 – 0.49) with a p-value of <0.001. In the obese group we observed a notable elevation (p= 0.021) of QUICKI from 0.33 (0.31 – 0.35) to 0.36 (0.32 – 0.39), as well as in the T2DM study cohort where the QUICKI increased form 0.31 (0.30 – 0.33) to 0.32 (0.31 – 0.37) with a p value of 0.001.

Fasting beta cell function

HOMA-beta 12 vs 36h: The HOMA-beta index is a measure of the activity level of pancreatic beta cells given in percentages. The fasting steady state of insulin secretion (beta cell function) did not show any significant changes. No changes in Beta-Cell function were observed when assessed with the HOMA-beta.

3.2 Fasting Hormone Levels

Fasting cortisol: The 36 hour fasting period showed a significant elevation in fasting cortisol levels in healthy (12h: 153.4 ± 68.3 vs 36h: 189.0 ± 59.3 ng/mL; p= 0.005) and T2DM (137.1 (90.3 - 155.0) vs 139.2 (101.4 - 215.1)ng/mL; p= 0.012) groups. Cortisol AUC levels have increased considerably only in the T2DM group (17438 ± 4846 vs. 21158 ± 5482; p=0.008).

Fasting Leptin: Blood serum levels of Leptin were notably decreased in all three groups after prolonged fasting. Healthy (1.9 (0.8 - 4.2) vs. 0.7 (0.2 - 1.0); p=0.000), Obese (16.5±10.2 vs. 12.7±10.9; p= 0.004) and Type2 Diabetes Mellitus (6.3 (4.5 - 13.4) vs. 2.1 (1.6 – 8.2) p=0.000).

Fasting free triiodothyronine: A significant increase in free triiodothyronine could be observed in both healthy (15.4 ± 2.4 vs. 17.0 ± 2.3; p=0.000) and obese (16.6 ± 2.1 vs. 17.7 ± 2.3; p=0.002) study populations after the longer fasting period. No such change was observed in people with type 2 diabetes (p=0.51).

Fasting free thyroxine: No relevant changes were seen in any groups in fasting free thyroxine levels.

Fasting adiponectin: No relevant changes were seen in any groups in fasting adiponectin levels.

3.3 Fasting Body Composition

Fat Mass: No effects of prolonged fasting were noted in the percental change of Fat mass nor in absolute fat mass measured in kilograms.

Body cell mass (kg and liter): BCM containing intracellular water and visceral protein did not show any difference after 36 hours of fasting.

Fat Free Mass: FFM dropped in all three study groups significantly as did the BMI.

Total Body water: An evident diminishing of the total body water after 36 hours of absolute calorie restriction was consistent throughout all study groups: Healthy (39.2 ± 8.4 vs. 37.6 ± 8.0; p<0.001), Obese (47.9 (44.2 - 50.5) vs. 46.7 (42.7 - 50.7); p= 0.025) and T2DM (46.3 ± 9.9 vs. 45.2 ± 9.6; p= 0.002)

BMR: The Basic metabolic rate decreased significantly after prolonged fasting in all three groups.

Water Balance and Hydrations Index: Fluid household was depleted remarkably by fasting for 36 hours, as the water balance and hydrations index dropped in both healthy and obese. Subjects with type 2 diabetes did not show comparable effects.

3.4 Postprandial Glucose Metabolism

3.4.1 Plasma Glucose 120min post OGTT

120minutes after ingestion of 75g of sugar the plasma glucose levels in the non-obese group did show a significant (p= 0.001) increase from 79.4 ± 18.4 when having fasted for 12 hours to 108.7 ± 31.0 mg/dL after 36 hours of fasting. The obese participants exhibited only an indicative increase in PG when comparing the results of short term to long term fasting (p value 0.067). In the T2DM study group no significant changes in the 120min PG levels could be shown.

3.4.2 Glucose and insulin response (AUC)

After the oral administration of 75mg of glucose the plasma glucose and serum insulin levels were monitored at timepoints 0, 15, 30, 60, 120 and 180 min within the 3-hours timespan. The glucose AUC in the non-obese cohort was significantly greater after the intervention of 36 hours of fasting compared to 12 hours of fasting (12h: 17070 ± 3128 mg/dL vs 36h: 21627 ± 4002 mg/dL; $p<0.001$. In the obese and Type 2 DM groups the postprandial glucose AUC was not significantly changed after 36h of fasting. Obese subjects showed following glucose AUC values: after a12h fast: 22638±3872 mg/dL vs after a 36h fast: 23315±3622 mg/dL with p=0.196. The cohort including people with type 2 diabetes exhibited a glucose AUC of 46771±11532 mg/dL following overnight fasting and 46238±7822 mg/dL after the prolonged fasting period with a p-value of 0.734.
Changes in insulin AUC in Healthy, obese or people with type 2 diabetes were not significant.

3.4.3 Insulin sensitivity:

The Indices ISI and Matsuda were used to assess the postprandial insulin sensitivity. The Matsuda index describes the pancreas' capacity to meet the necessary insulin secretion rate to level out an individual's insulin resistance, with reference values lying within 0-12 and below 4.3 being predictable for Insulin resistance (15). Healthy subjects had a significant

increase after 36 hours of fasting from 12.8 ± 9.5 (12 hours fast)to 18.8 ± 13.4 (p=0.004) as well as subjects with type 2 diabetes who grew from 2.6 (2.2 – 4.8) following an overnight fast to 3.0 (2.0 – 6.8) after the prolonged fasting (p= 0.014). However, the obese group did not show any significant effect concerning the Matsuda insulin sensitivity index. No significant changes were seen in any group regarding the ISI.

3.4.4 Beta cell function:

Stumvoll Index encompassing 1st and 2nd phase of insulin secretion and the OGTT ratio were used to determine the beta cell function. We observed a significant decrease in 1st (1138 ± 496 vs 742 ± 471 ; p= 0,001) and 2nd (295 ± 108 vs 211 ± 104 p= 0,001) phase insulin secretion only in the non-obese study population after ingestion of the 75g of glucose. Obese and participants with type 2 diabetes did not show any significant changes regarding the two insulin secretion phases. Only in the non-obese group, OGTT ratios following the 36h fasting period resulted in an impressive reduction compared to the 12 hours of fasting (12h fasting: 1.14 (0.95 - 1.50) vs 36h of fasting: 0.49 (0.13 - 0.86), p=0,001). No such alterations in OGTT ratios after 36h of fasting could be shown in either the obese or the T2DM group.

Table 1 The effect of prolonged fasting (36h) versus overnight fasting (12h) on fasting glucregulatory markers in healthy, obese and subjects with type 2 diabetes.

	Healthy			Obese			Type 2 diabetes		
	After 12h fasting	After 36h fasting	p-value	After 12h fasting	After 36h fasting	p-value	After 12h fasting	After 36h fasting	p-value
Fasting Glucoegulatory Parameters									
Fasting Glucose [mg/dL]	80.3 ± 7.3	73.4 ± 10.6	**0.003**	93.7 ± 9.9	87.5 ± 12.6	**0.002**	149.9 ± 36.9	135.7 ± 26.3	**0.017**
Fasting insulin [mU/L]	4.3 (4.0 - 7.1)	2.5 (1.8 - 4.1)	**0.002**	11.4 (6.7 - 15.9)	7.2 (5.0 - 14.5)	**0.020**	11.2 ± 4.4	9.2 ± 5.8	**0.007**
Fasting C-peptide [ng/mL]	0.94 (0.83 - 1.13)	0.61 (0.43 - 0.82)	**0.001**	2.00 (1.55 - 2.46)	1.65 (0.95 - 2.24)	**0.036**	2.31 ± 0.75	1.94 ± 1.02	**0.002**
QUICKI	0.39 (0.37 - 0.40)	0.45 (0.41 - 0.49)	**0.000**	0.33 (0.31 - 0.35)	0.36 (0.32 - 0.39)	**0.021**	0.31 (0.30 - 0.33)	0.32 (0.31 - 0.37)	**0.001**
HOMA-IR	0.86 (0.75 - 1.41)	0.43 (0.27 - 0.67)	**0.001**	2.52 (1.72 - 4.27)	1.37 (0.90 - 3.07)	**0.001**	4.26 ± 2.06	3.17 ± 2.17	**0.012**
Proinsulin [pmol/L]	3.9 (3.4 – 5.0)	3.6 (2.7 – 4.6)	0.111	9.9 (7.0 – 25.9)	11.1 (6.6 – 16.6)	0.151	23.6 (15.3 – 37.1)	20.7 (10.6 – 33.0)	**0.019**
HOMA-β	105.4 (75.9 - 161.7)	63.6 (18.4 - 175.9)	0.202	140.3 (89.1 - 240.0)	149.1 (82.3 - 252.9)	0.963	53.2 ± 31.8	47.1 ± 32.4	0.102

Table 2 The effect of prolonged fasting (36h) versus overnight fasting (12h) on postprandial glucregulatory markers in healthy, obese and subjects with type 2 diabetes.

	Healthy			Obese			Type 2 diabetes		
	After 12h fasting	After 36h fasting	p-value	After 12h fasting	After 36h fasting	p-value	After 12h fasting	After 36h fasting	p-value
Postprandial Glucoegulatory Parameters									
PG 120 min [mg/dL]	79.4 ± 18.4	108.7 ± 31.0	**0.001**	108.0 ± 35.5	116.7 ± 29.0	0.067	261.8 ± 83.1	281.5 ± 58.5	0.121
Glucose [mg/dl] AUC	17070 ± 3128	21627 ± 4002	**0.000**	22638 ± 3872	23315 ± 3622	0.197	46771 ± 11532	46238 ± 7822	0.734
Insulin [mU/L] AUC	5592 ± 2679	7557 ± 5448	0.137	12621 ± 8364	13794 ± 9254	0.629	7695 ± 4427	8337 ± 4394	0.392
ISI	0.13 (0.13 - 0.13)	0.13 (0.13 - 0.13)	0.109	0.09 (0.08 - 0.10)	0.09 (0.08 - 0.10)	0.125	0.09 (0.07 - 0.10)	0.09 (0.07 - 0.11)	0.750
1st phase insulin secretion	1138 ± 496	742 ± 471	**0.001**	1400 ± 956	1408 ± 759	0.972	-68 ± 635	-17 ± 585	0.536
2nd phase insulin secretion	295 ± 108	211 ± 104	**0.001**	370 ± 224	370 ± 176	0.994	68 ± 130	73 ± 125	0.740
$\Delta Ins_{30}/\Delta Glc_{30}$	1.14 (0.95 - 1.50)	0.49 (0.13 - 0.86)	**0.001**	0.83 (0.50 - 1.91)	0.92 (0.47 - 2.11)	0.762	0.23 ± 0.23	0.21 ± 0.22	0.157
Matsuda Index	12.8 ± 9.5	18.8 ± 13.4	**0.004**	4.0 (3.2 - 5.3)	5.4 (2.7 - 7.2)	0.078	2.6 (2.2 - 4.8)	3.0 (2.0 - 6.8)	**0.014**

Table 3 The effects of 36 hours fasting compared to 12 hours of fasting on the body composition in healthy, obese, and people with type 2 diabetes.

	Healthy			Obese			Type 2 Diabetes		
	After 12h fasting	After 36h fasting	p-value	After 12h fasting	After 36h fasting	p-value	After 12h fasting	After 36h fasting	p-value
BODY COMPOSITION									
Fat mass [%]	21.4 (17.0 – 25.7)	20.2 (17.0 – 24.5)	0.369	38.6 ± 9.4	39.0 ± 9.0	0.337	33.6 ± 10.3	33.7 ± 10.1	0.664
Fat mass [kg]	14.0 (11.7 - 16.9)	14.2 (11.5 - 16.1)	0.054	41.1 ± 14.5	41.1 ± 14.1	0.984	34.0 ± 18.6	33.7 ± 18.2	0.317
BMI [kg/m2]	22.9 ± 1.5	22.5 ± 1.4	**0.000**	32.9 (31.3 - 36.4)	32.7 (30.9 - 36.2)	**0.000**	29.4 (27.0 - 34.6)	29.1 (26.7 - 34.3)	**0.002**
BMR [kcal]	1633.7 ± 238.0	1619.5 ± 231.5	**0.000**	2051.3 (1730.8 - 2201.0)	2031.4 (1718.3 - 2172.4)	**0.000**	1791.6 ± 402.0	1775.8 ± 397.1	**0.000**

Table 4 The effects of 36 hours fasting compared to 12 hours of fasting on hormone levels in healthy, obese and people with type 2 diabetes.

	Healthy			Obese			Type 2 Diabetes		
	After 12h fasting	After 36h fasting	p-value	After 12h fasting	After 36 h fasting	p-value	After 12h fasting	After 36h fasting	p-value
HORMONES									
Free trijodthyronine [pmol/L]	15.4 ± 2.4	17.0 ± 2.3	**0.000**	16.6 ± 2.1	17.7 ± 2.3	**0.002**	16.1 (15.3 - 17.4)	17.0 (15.8 - 19.0)	0.051
Leptin [ng/mL]	1.9 (0.8 - 4.2)	0.7 (0.2 - 1.0)	**0.000**	16.5±10.2	12.7±10.9	**0.004**	6.3 (4.5 - 13.4)	2.1 (1.6 – 8.2)	**0.000**
Fasting cortisol [ng/mL]	153.4 ± 68.3	189.0 ± 59.3	**0.005**	115.2 ± 67.4	123.0 ± 53.2	0.478	137.1 (90.3 - 155.0)	139.2 (101.4 - 215.1)	**0.012**
Cortisol [ng/mL] AUC (in minutes)	21071 ± 11165	23562 ± 8730	0.282	14634 ± 5250	17217 ± 6795	0.119	17438 ± 4846	21158 ± 5482	**0.008**

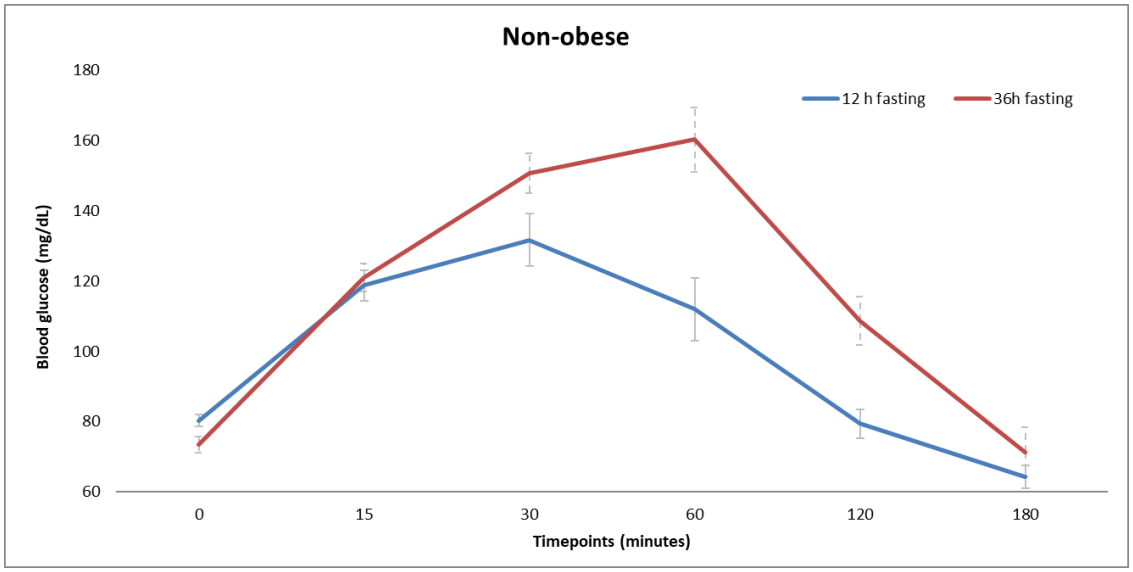


Figure 7 - Postprandial blood glucose levels in healthy subjects

Blood glucose levels after a 75g oral glucose tolerance test in healthy subjects following 12h (blue line) and 36h (red line) of fasting. This group was the only to produce significant results in glucose AUC. It seems that the maximum PG-level peaked at 60 minutes (160,3 mg/dL) on the 36h-curve whereas the 12h-curve shows its maximum earlier at 30 min (131,7 mg/dL). The decrease after each curve's peak is steeper for the 36h-curve.

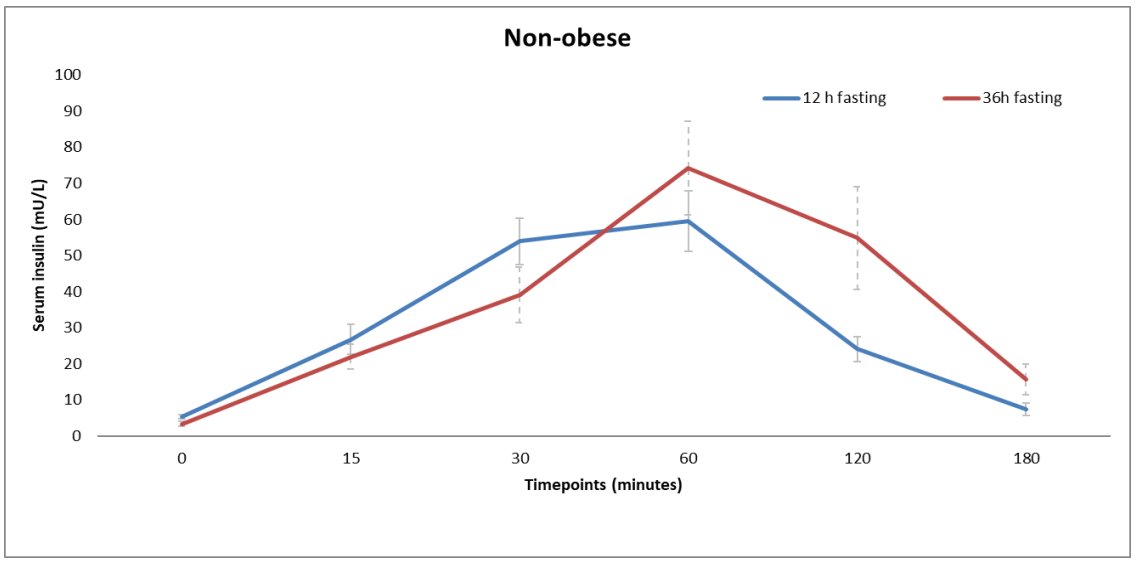


Figure 10 - Postprandial insulin response in healthy subjects

Insulin response in non-obese participants after 12 hours (blue line) and 36 hours (red line) of fasting. On the one hand we observe two peaks of insulin at timepoints 30min and 60min on the 12h-curve, with a following steady decrease. The 36h curve on the other hand appears to lack the 30min peak, however exhibiting a higher peak compared to the 12h-curve at 60 min post OGTT. This would explain the higher blood glucose implied by the 36h-curve at 30min and the exaggerated peak at 60 min compared to the 12h-curve in Figure 1.

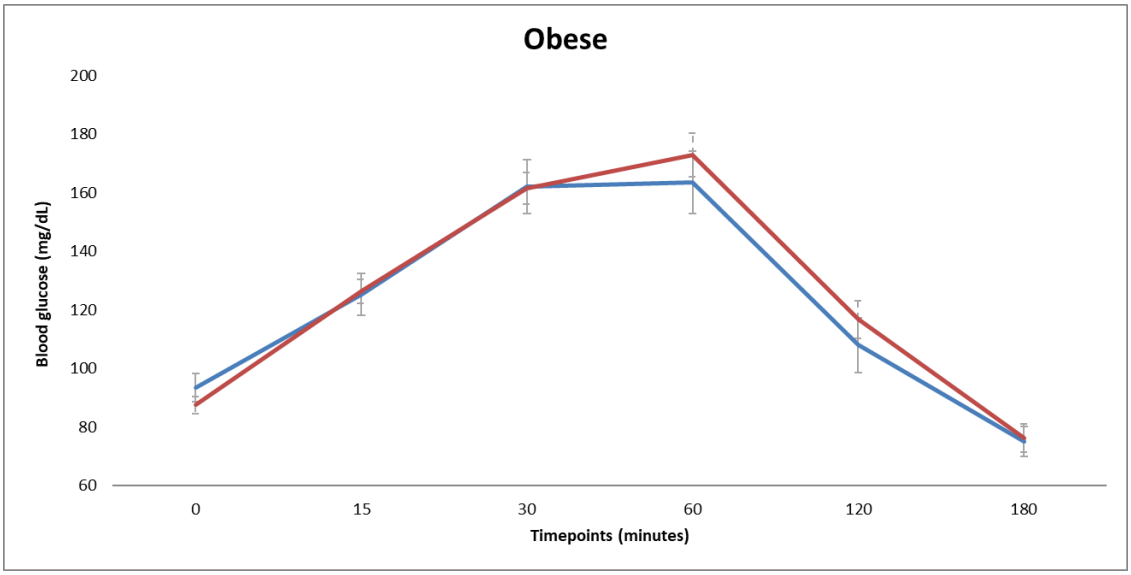
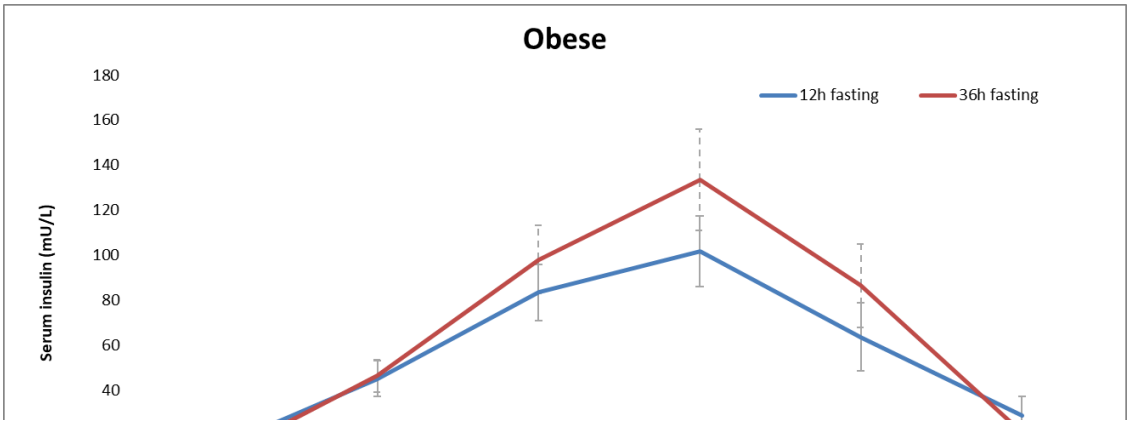


Figure 13 - Postprandial blood glucose levels in obese subjects

Postprandial blood glucose in obese subjects following 12 hours (blue line) and 36 hours (red line) of total energy restriction. Curve progressions after 12h and 36h of fasting for glucose AUC in obese are similar and almost align during the first 30 minutes post-OGTT. At 60min the 36h-curve has a slightly higher peak than the 12h curve with 172.9 mg/dL and 163.5 mg/dL respectively, before the PG starts to drop for both curves at circa the same rate.



Insulin response in obese participants after 12 hours (blue line) and 36 hours (red line) of fasting. The 36h-curve rises steeper from 15min onwards having a higher peak value (133.4 mU/L) at 60min compared to the 12-h curve (101.8 mU/L). More Insulin is needed to effectively dispose the blood glucose after 36 h indicating a slight, yet non- significant insulin resistance caused by fasting in obese subjects.

Insulin response in obese participants after 12 hours (blue line) and 36 hours (red line) of fasting. The 36h-curve rises steeper from 15min onwards having a higher peak value (133.4 mU/L) at 60min compared to the 12-h curve (101.8 mU/L). More Insulin is needed to effectively dispose the blood glucose after 36 h indicating a slight, yet non- significant insulin resistance caused by fasting in obese subjects.

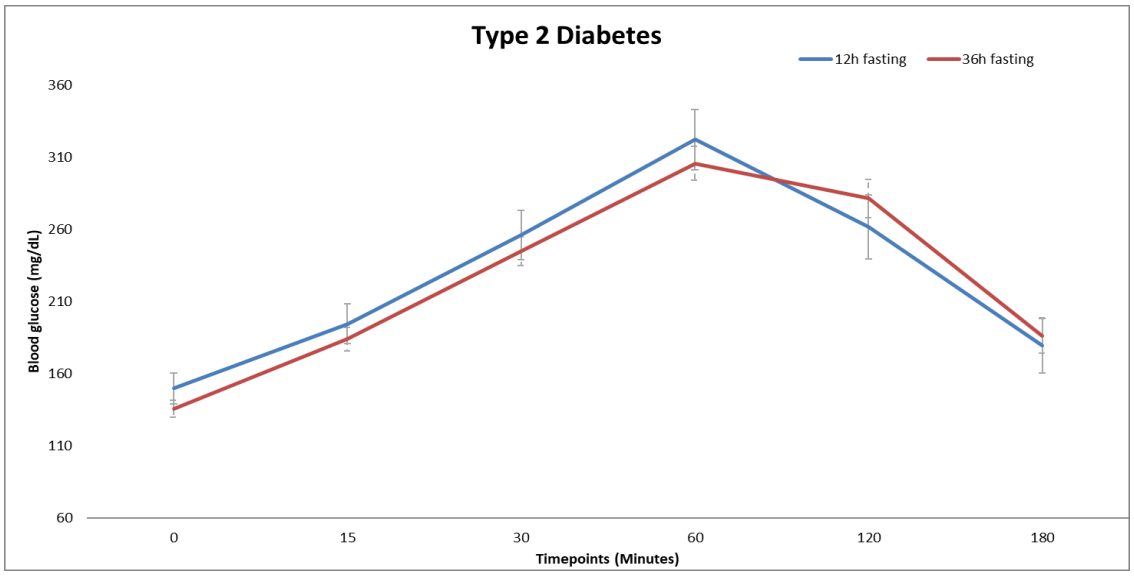


Figure 19 - Postprandial blood glucose levels in subjects with type 2 diabetes

Postprandial blood glucose in people with type 2 diabetes following 12 hours (blue line) and 36 hours (red line) of total energy restriction. In study subjects with type 2 diabetes the two curves progress almost parallel to each other until the 60minute mark with the 12h-curve showing a slightly higher peak (322.4 mg/dL) than the 36h-curve (305.9 mg/dL). Both curves start to descend surpassing the 60-minute mark with the 12h-curve following a slightly steeper course than the 36h-curve.

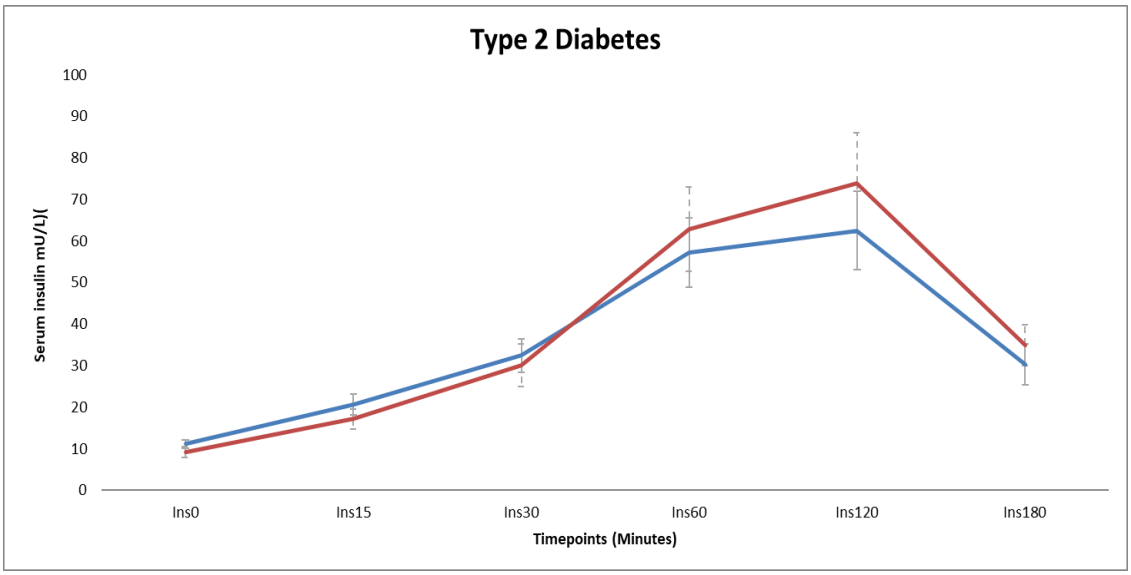


Figure 22 - Postprandial insulin response in subjects with type 2 diabetes

Insulin response in people with type 2 diabetes after 12 hours (blue line) and 36 hours (red line) of fasting. The course of the insulin curves is similar after reaching 30 minutes post-OGTT. After that we see a stronger increase in serum insulin levels for the 36h-curve seemingly peaking at 120min (73.9 mU/L). The 12h curve also exhibits a peak at 120min (62.4 mU/L) however with non-significant lower serum insulin levels. This suggests a delay of the insulin peak to 120 min.

4 Discussion

4.1 Changes in fasting glucose metabolism

In this dietary intervention study, we observed a significant elevation in fasting insulin sensitivity in all three cohorts (healthy, obese, and Type 2 Diabetes) after 36 hours of fasting compared to 12 hours of fasting. HOMA-IR and QUICKI are two very useful mathematical models to approximate fasting insulin sensitivity and have been found to highly correlate in their results, granting a higher potency upon the interpretation of our study results.(17)

We used HOMA-IR and QUICKI as fasting insulin sensitivity indices taking into calculation the fasting glucose and fasting insulin levels. HOMA-IR and QUICKI are giving information about the steady state of glucose homeostasis balanced by hepatic glucose output and insulin secretion regulated by a feedback mechanism between the beta cells and the liver (15). On the one hand a low HOMA-IR value reflects low insulin and glucose levels, indicating a high insulin sensitivity because only a small amount of insulin is needed to maintain the glucose homeostasis. On the other hand, a low QUICKI value speaks for a higher insulin resistance. In our study the healthy group demonstrated a remarkable drop of HOMA-IR values, following 12 hours and 36 hours of fasting, respectively. A study performed by Horne et al. examining two healthy study populations, having the intervention group fasting for 28 hours showed a similar drop in HOMA-IR, whereas the normally fed control group did not exhibit any changes in insulin sensitivity.(18) Further, total energy restriction (100%) seems to be more effective than partial caloric restriction, as insulin sensitivity did not significantly increase in healthy subjects who were allowed energy intake on fast days. (18) (19)

In our obese study cohort, the HOMA-IR also significantly dropped after the 36 hours of total caloric restriction. Similar results are seen in obese individuals fasting daily over 30 days for 15 hours during Ramadan, as the HOMA-IR is significantly reduced. (20) Other studies examined the effects of daily calorie restrictions with the goal of weight loss in obese and overweight populations on insulin sensitivity. Kim et al's study demonstrated that a daily CR of circa 700kcal for 3 months in obese men spawns a significant ($p=0.006$) reduction in HOMA-IR and an increase in QUICKI. (21) On the other hand Hietaniemi et al. did not reach significance ($p=0.052$) in their study of obese middle-aged women performing a CR-diet ingesting only 1200kcal per day over 2. (22) The lack of significance

in HOMA-IR of the study of Hietaniemi et al. compared to the one of Kim MK et al. could be explained by the higher percentual weight loss in Kim et al's study (Kim et al. 12% and Hietaniemi 5 % weight loss) as two studies prove a positive correlation between HOMA-IR and BMI.(23) (24) Contrary results were obtained by Razny et al. demonstrating no significant increase in insulin sensitivity (HOMA-IR) after putting obese male and female subjects on a hypocaloric diet, however when supplemented with n-3 polyunsaturated fatty acids a notable elevation in insulin sensitivity was found ($p=0.01$). (25)

Prior data on total energy restriction over a prolonged time interval in people with type 2 diabetes is limited, however there are some studies examining the effects of severe energy restriction. Gabel et al were able to demonstrate that alternate day fasting in obese, insulin-resistant individuals (BMI, mean= 34 kg/m²) over 12 months with an energy intake of only 25% from the basic metabolic rate on fasting days and 125% on feasting days lead to a significant decrease of the HOMA-IR ($p<0.05$).(6) It seems vital that a severe energy restriction is being performed as calorie restriction to a total of 75% of BMR (= -25 % of calorie intake per day) per day shows a similar but considerably diminished effect on HOMA-IR and other fasting/ IR parameters (6). This effect is supported by the results of the DiRECT study, showing a positive correlation between the extend of weight reduction and the ratio of diabetes remission since 34% with a 5-10 kg, 57% with a 10-15 kg and 86% with an >15 kg weight loss were able to get rid of their antidiabetic medication.(26)

Another study confirming the reduction of insulin resistance in people with type 2 diabetes included three insulin dependent individuals with type 2 diabetes, who were able to discontinue their antidiabetic medication following a 24-h alternate day fasting regime over 7 to 11 months. (4)

We can conclude that the insulin sensitivity increases during fasting in healthy, obese, and patients with type 2 diabetes, which is consistent with the present research. Our study results align with the already existing studies indicating, the longer the fasting periods are the more the insulin resistance decreases, that is true for all three study populations. Also, a 100% energy restriction is more likely to increase insulin sensitivity, whereas CR allowing a small daily caloric intake needs to be performed over a longer period to gain the same effect. Weight loss is another predictor for ameliorated glucose tolerance, as our three study groups show a remarkable drop in BMI after the prolonged fasting intervention. As described above, comparable results have been described in fasting studies including obese and people with type 2 diabetes.(23)(24)

4.2 Postprandial changes

Aside investigating the changes of glucose metabolism after fasting, we also put the focus on the postprandial alterations of glucose tolerance. The results after administering the OGTT following the prolonged fasting period (36h) show a significant elevation of the glucose AUC and PG 120 minutes after glucose uptake in healthy subjects when compared to an overnight fast (12h). The same parameters were slightly increased however not significantly in obese and no remarkable changes were notable in subjects with diabetes. These findings suggest a fasting induced glucose disposal impairment in healthy adults after a prolonged fasting period, which is consistent with previously published results on prolonged fasting. (27) High FFA levels that occur during prolonged fasting exert an inhibitory effect on pyruvate dehydrogenase and thereby impairing the insulin mediated glucose uptake, which may play a role in postprandial glucose level increase in healthy subjects. (28) It is evident that the body prioritizes fat oxidation over glucose oxidation after a long fasting period, which is consistent with previous finding (29)(30). Studies suggest that this mechanism ensures the renewal of muscle glycogen stores rather than generating immediate energy by oxidizing the newly ingested glucose since energy supply is already being upheld by fat oxidation.(31) In summary the 2-h post OGTT PG levels in healthy subjects are higher after 36h vs 12h of total ER because disposal of glucose mainly happens through glycogen repletion leaving out glucose oxidation. Shulman et al proposed another mechanism by which increased FFA-levels hinder the postprandial glucose uptake by causing a defective GLUT-4 integration into the cell surface. (32) In addition we found that insulin secretion in healthy subjects after prolonged fasting is diminished but peaks at the same time as after overnight fasting at 60 minutes post OGTT, while no significant change of the integrated postprandial insulin secretion was detectable. This is indicated by the significant decrease in Stumvoll 1^{st} and 2^{nd} phase of insulin secretion and $OGTT_{ratio}$ which represent the postprandial beta cell function (table 2). The $OGTT_{ratio}$ demonstrates a remarkable postprandial glucose disposal impairment after 36h of fasting in healthy. These findings are coherent with previously published data and indicate a transient beta cell function defect in healthy subjects, while in people with type 2 diabetes this beta cell impairment is permanent .(33) It is known that during extended fasting FFA-cycle- and glycolysis derived coupling factors are depleted as glucose oxidation is slowed down and FFA's are used for beta cells energy supply, consuming coupling factors substrates.(9) These coupling factors play a major role for the amplifying pathway of insulin

secretion,(34) however their scarcity after prolonged fasting must be compensated by an enhanced glucose and fatty acid oxidation possibly explaining the delay in adequate insulin excretion. This impairment in beta cell activity can also be linked to the notable increase in fasting cortisol levels after 36h fasting inhibiting insulin secretion and leading to postprandial insulin resistance.(35)(36) The impairment in postprandial glucose disposal in healthy individuals might be due to a decrease in beta cell activity, glucose oxidation and cortisol induced insulin resistance. Obese participants had a slightly greater, however insignificant glucose AUC and insulin AUC after prolonged fasting compared to 12 hours of fasting (figure 5 & 6). In contrast Antoni et al demonstrated that in a group of obese subjects (mean BMI(kg/m²) = 29.1, SEM= 0.8), those who underwent 100% ER for 36h had a significant increase in glucose AUC compared to subjects who were following an isoenergetic diet performing an overnight fast (12h). (27) Interestingly insulin AUC did not show any significant changes in the study of Antoni et al, similar to our results. This study suggests that the glucose disposal in obese is significantly less efficient after 36 hours of fasting compared to 12 hours of fasting, because comparable postprandial insulin levels are observed after both interventions. In conclusion, obese individuals' insulin secretion phases are not significantly altered by prolonged fasting because of their constant overproduction of insulin, however the slight elevation in glucose AUC after 36h fasting can be linked to an insulin resistance as indicated by prior studies.(27) Obese participants did not show cortisol fluctuations after fasting for 36 hours, thereby not diminishing the insulin secretion as in healthy subjects. Participants with type 2 diabetes showed no postprandial changes as glucose AUC and insulin AUC showed the same course comparing 36 and 12 h of fasting. It seems that insulin production is delayed with the peak at 120 min post OGTT. This delay might be due to a beta cell impairment and consistent with prior data.(37)(38) In contrast to our findings Duska et al found that a 60 hour long fast leads to a worsened glucose disposal in people with type 2 diabetes and obese individuals.(39)

In conclusion this study shows that prolonged fasting for 36 hours leads to an increase in fasting insulin sensitivity in healthy, obese, and subjects with type 2 diabetes. Further, we showed that a total energy restriction for 36 hours leads to a defective postprandial glucose disposal caused by a delayed insulin production in healthy participants, a slight however non-significant increase in plasma glucose levels in obese and no changes in postprandial glucose metabolism in people with type 2 diabetes. A metabolic switch seems to be causing beta cell activity decrease in healthy, however not in obese and people with type 2

diabetes since their beta cells are active all the time. A one-time prolonged fasting period of 36 hours does not seem to have any effect on postprandial glucose disposal in obese or in people with type 2 diabetes, yet an increasing effect in healthy subjects. Halberg et al. demonstrated that alternate day fasting encompassing 20 hours of continuous fasting over two weeks leads to a more effective glucose uptake in healthy men. (40). These results compared to our findings may give a hint of the optimal duration of fasting, however to gain a better understanding more research must be done investigating the effects of fasting on glucose disposal in obese and people with type 2 diabetes.

5 References

1. Heilbronn LK, Smith SR. Alternate-day fasting in nonobese subjects: Effects on body weight, body composition, and energy metabolism. Am J Clin Nutr. 2005;81(1):69–73.
2. Patterson RE, Sears DD. Metabolic Effects of Intermittent Fasting. Annu Rev Nutr 2017 3761–623. 2017;(May):1–23.
3. Corley BT, Carroll RW. Research: Treatment Intermittent fasting in Type 2 diabetes mellitus and the risk of hypoglycaemia: a randomized controlled trial. 2018;0–2. Available from: doi: 10.1111/dme.13595
4. Furmli S, Elmasry R. Therapeutic use of intermittent fasting for people with type 2 diabetes as an alternative to insulin. BMJ Case Rep. 2018;2018:1–5.
5. Li C, Sadraie B. Effects of A One-week Fasting Therapy in Patients with Type-2 Diabetes Mellitus and Metabolic Syndrome - A Randomized Controlled Explorative Study. Exp Clin Endocrinol Diabetes. 2017;125(9):618–24.
6. Gabel K, Kroeger CM. Differential Effects of Alternate-Day Fasting Versus Daily Calorie Restriction on Insulin Resistance. Obesity. 2019;27(9):1443–50.
7. Hoddy KK, Gibbons C. Changes in hunger and fullness in relation to gut peptides before and after 8 weeks of alternate day fasting. Clin Nutr. 2016;6–11.
8. Bhutani S, Klempel MC. Alternate Day Fasting and Endurance Exercise Combine to Reduce Body Weight and Favorably Alter Plasma Lipids in Obese Humans. Obesity. 2013;21(7).
9. Wortham M, Sander M. Mechanisms of β-cell functional adaptation to changes in workload. Diabetes Obes Metab. 2017;18(Suppl 1):78–86.
10. Röder P V., Wu B. Pancreatic regulation of glucose homeostasis. Exp Mol Med. 2016;48(November 2015):e219.
11. Cherkas A, Golota S. An intermittent exhaustion of the pool of glycogen in the human organism as a simple universal health promoting mechanism. Med Hypotheses. 2014;82(3):387–9.
12. Cahill GF. Fuel Metabolism In Starvation. Annu Rev Nutr. 2006;26:1–22.
13. Leighton E, Ar C, Gregory S. A Practical Review of C-Peptide Testing in Diabetes WHAT IS C-PEPTIDE AND WHY MIGHT IT BE USEFUL IN CLINICAL PROBLEMS WITH C-PEPTIDE. Diabetes Ther. 2017;8(3):475–87.
14. Article O. Indices of insulin action calculated from fasting glucose and insulin reflect hepatic , not peripheral , insulin sensitivity in African- American and Caucasian adolescents. 2008;9(Part II):57–61.
15. Gutch M, Kumar S. Assessment of insulin sensitivity / resistance. Indian J Endocrinol Metab. 2015;19(1).
16. Tohidi M, Ghasemi A, Hadaegh F, Derakhshan A. Age- and sex-speci fi c reference values for fasting serum insulin levels and insulin resistance / sensitivity indices in healthy Iranian adults : Tehran Lipid and Glucose Study. Clin Biochem [Internet]. 2014;47(6):432–8. Available from: http://dx.doi.org/10.1016/j.clinbiochem.2014.02.007
17. Goyal G, Panag KMS. Correlation of insulin resistance by various methods with fasting insulin in obese. Int J Appl Basic Med Res. 2014;4(3):41.
18. Horne BD, Muhlestein JB. Randomized cross-over trial of short-term water-only fasting: Metabolic and cardiovascular consequences. Nutr Metab Cardiovasc Dis. 2013;23(11):1050–7.
19. Carlson O, Martin B. Impact of reduced meal frequency without caloric restriction

on glucose regulation in healthy, normal-weight middle-aged men and women. Metabolism. 2007;56(12):1729–34.
20. Aksungar FB, Sarikaya M. Comparison of intermittend fasting versus caloric restriction in obese subjects: A two year follow-up. J Nutr Heal AGING©. 2016;
21. Kim MK. Effects of low calorie diet-induced weight loss on post-exercise heart rate recovery in obese men. J Exerc Nutr Biochem. 2014;18(2):181–8.
22. Hietaniemi M, Jokela M. The effect of a short-term hypocaloric diet on liver gene expression and metabolic risk factors in obese women. Nutr Metab Cardiovasc Dis. 2009;19(3):177–83.
23. Otake T, Fukumoto J. Linking lifestyle factors and insulin resistance, based on fasting plasma insulin and HOMA-IR in middle-aged Japanese men: A cross-sectional study. Scand J Clin Lab Invest. 2014;74(6):536–45.
24. Martinez KE, Tucker LA. Expanded Normal Weight Obesity and Insulin Resistance in US Adults of the National Health and Nutrition Examination Survey. J Diabetes Res. 2017;Article ID:8 pages.
25. Razny U, Kiec-Wilk B. Effect of caloric restriction with or without n-3 polyunsaturated fatty acids on insulin sensitivity in obese subjects: A randomized placebo controlled trial. BBA Clin. 2015;4:7–13.
26. Lean ME, Leslie WS. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. Lancet. 2018;391(10120):541–51.
27. Antoni R, Johnston KL. Investigation into the acute effects of total and partial energy restriction on postprandial metabolism among overweight/obese participants. Br J Nutr. 2016;115(6):951–9.
28. Fery F, D'Attellis NP. Mechanisms of starvation diabetes: A study with double tracer and indirect calorimetry. Am J Physiol - Endocrinol Metab. 1990;259(6 22-6).
29. Balasse E 0., Neef MA. Operation of the "Glucose-Fatty Acid Cycle" during Experimental Elevations of Plasma Free Fatty Acid Levels in Man. Eur J Clin Invest. 1974;252:247–52.
30. Hultman E, Bergström J. Muscle Glycogen Synthesis in Relation to Diet Studied in Normal Subjects. Acta Med Scand. 1967;182(1).
31. Randle PJ, Kerbey AL. Mechanisms Decreasing Glucose Oxidation in Diabetes and Starvation: Role of Lipid Fuels and Hormones. Diabetes Metab Rev. 1988;4(7):623–38.
32. Roden M, Price TB. Mechanism of Free Fatty Acid – induced Insulin Resistance in Humans. J Clin Invest. 1996;97(12):2859–65.
33. Kramer CK, Vuksan V. Emerging parameters of the insulin and glucose response on the oral glucose tolerance test: Reproducibility and implications for glucose homeostasis in individuals with and without diabetes. Diabetes Res Clin Pract. 2014;105(1):88–95.
34. Prentki M, Matschinsky FM. Metabolic Signaling in Fuel-Induced Insulin Secretion. Cell Metab. 2013;18(2):162–85.
35. Plat L, Byrne MM. Effects of morning cortisol elevation on insulin secretion and glucose regulation in humans. Am J Physiol - Endocrinol Metab. 1996;270(1 33-1):36–42.
36. Horton TJ, Hill JO. Prolonged fasting significantly changes nutrient oxidation and glucose tolerance after a normal mixed meal. J Appl Physiol. 2001;90(1):155–63.
37. Basu A, Dalla Man C. Effects of type 2 diabetes on insulin secretion, insulin action, glucose effectiveness, and postprandial glucose metabolism. Diabetes Care. 2009;32:866–72.
38. Garber AJ. The importance of early insulin secretion and its impact on glycaemic

regulation. Int J Obes. 2000;24(Suppl. 3):S32–7.
39. Duska F, Andel M. Effects of acute starvation on insulin resistance in obese patients with and without type 2 diabetes mellitus. Clin Nutr. 2005;24:1056–64.
40. Halberg N, Henriksen M. Effect of intermittent fasting and refeeding on insulin action in healthy men. J Appl Physiol. 2005;99(6):2128–36.