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Effects of different milk proteins on the metabolic response in healthy and prediabetic volunteers

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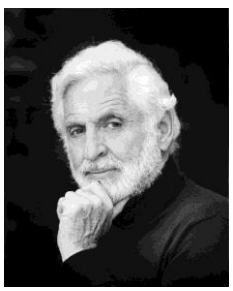
A hypothesis is a sleeping beauty
waiting for the prince to wake it.
The prince is the proving experiment.
(Carl Djerassi 1923-2015,
Inventor of the birth control pill)



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Abbreviations

°C	Celsius
µmol	Micromole
AUC	Area under the curve
BCAA	Branched chain amino acid
BMI	Body-mass-index
C	Control group (Healthy group)
cAMP	Cyclic adenosine monophosphate
CaSR	Calcium-sensing receptor
cCGP	Caseinoglycopeptide
CCK	Cholecystokinin
Cm	Centimeter
CMP	Caseinmacropeptide
CP	Casein protein (Sodium caseinate)
C-peptide	Connecting peptide
DACH	Deutschland, Österreich, Schweiz
DGE	Deutsche Gesellschaft für Ernährung
Dl	Deciliter
DPP-4	Dipeptidyl peptidase -4
DRKS	Deutsches Register für Klinische Studien
E%	Energy%
EAA	Essential amino acids
EDTA	Ethylendiaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmatic reticulum
et al.	et alia
GIP	Glucose dependent insulinotropic polypeptide
GITT	Gastrointestinal transit time
GLP-1	Glucagon-like peptide 1
GLUT4	Glucose transporter 4
GMP	Glycomacropeptide
GOAT	Ghrelin O-acyltransferase
GSK	Glycogen synthase kinase
GSR	Glutathione peroxidase
HB	Hydroxybutyrate
HB_{A1c}	$Hemoglobin_{A1c}$
HMG-CoA	3-Hydroxy-3-methyl-glutaryl – Coenzyme A
HOMA	Homeostasis Model Assessment
HT	Hydroxytryptamin
IC_{50}	Concentration of an inhibitor with half maximal
IDF	International Diabetes Federation
IGF1	Insulin-like growth factor 1
IGFBP-1	Insulin-like growth factor binding protein 1
IL-6	Interleukine 6
Incretin	Intestine Secretion Insulin
IPA	Isoleucine-Proline-Alanine
IRS-1	Insulin receptor substrate 1
iTRAQ	Isobaric tag for relative and absolute

L	Liter
LAT1	L-type amino acid transporter 1
LC-MS/MS	L-type amino acid transporter 2
LNAA	Long neutral amino acids
M	Meter
MD	Maltodextrin
Min	Minute
MIPROMET	Milk protein metabolism
Mmol	Millimole
MOR	µ-Opioid receptors
Mosm	Milliosmole
MSH	Melanocortin-stimulating hormone
mTOR	Mammalian target of rapamycin
mTORC1	Mammalian target of rapamycin complex 1
mTORC2	Mammalian target of rapamycin complex 2
NaCl	Sodium chloride
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide hydride
NCD	Non communicable disease
NEFA	Non-esterified fatty acids
NH4Ac	Amonium acetate
OCTT	Oro-cecal transit time
OGTT	Oral glucose tolerance test
P	Presumption
P	Prediabetics
PBMC	Peripheral blood mononuclear cells
PC	Proconvertase
PDK	Protein dependent kinase
PEPT-1	Peptide transporter 1
Pg	Picogram
PI3-kinase	Phosphatidylinositol-3-kinase
PI3P	Phosphatidylinositol-3-phosphate
PIP_3	Phosphatidylinositol (3,4,5)-triphosphate
PKA	Proteinkinase A
PKB	Protein kinase B
PKU	Phenylketonuria
Pmol	Picomole
POMC	Pro-opiomelanocortin
PTFE	Polytetraflourethylene
PYY	Peptide tyrosine tyrosine, Pancreatic peptide
R	Correlation coefficient
R^2	Coefficient of determination of a linear
Rheb	Ras homolog enriched in brain
ROS	Reactive oxygen species
RQ	Respiratory Quotient
S6K1	p70 S6 kinase 1
SD	Standard deviation
SEM	Standard error of the mean

SOD	Superoxide dismutase
TAA	Total amino acids
T2R	Taste receptor type 2
TCA	Tricarboxylic acid cycle
TSC1	Tuberous sclerosis protein 1
TSC2	Tuberous sclerosis protein 2
UCP	Uncoupling Protein
VAS	Visual analogue scale
WHO	World health organization
WPI	Whey protein isolate
ZIEL	Zentralinstitut für Ernährung und Lebensmittel

Abbreviations of amino acids and amino acid derived products

Aad	α-Aminoadipate
Abu	α-Aminobutyrate
Ala	Alanine
Asn	Asparagine
Arg	Arginine
Cys	Cystine
Cit	Citrulline
Gln	Glutamine
Glu	Glutamate
Gly	Glycine
His	Histidine
Hyp	Hydroxyproline
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Orn	Ornithine
PetN	Phospho-ethanolamine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Tau	Taurine
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosin
Val	Valine

Abbreviations of Acylcarnitine species

C0	Free Carnitine
C2	Acetylcarnitine
C3	Propionylcarnitine
C4	Butyrylcarnitine
C5	Valerylcarnitine
C14	Tetradecanoylcarnitine
C16	Hexadecanoylcarnitine

Summary

In light of the obesity and diabetes prevalence there is growing interest in food components which can evoke a beneficial modulation in the glycemic response. Cow´s milk derived whey protein has received particular attention. It contains high amounts of branched chain amino acids (BCAAs) which are assumed to increase insulin secretion from ß-cells directly and indrectly via the glucose dependent insulinotropic polypeptide (GIP) and glucagon like peptide 1 (GLP-1) – so called incretins – secreted from enteroendocrine cells in the gut lineage. However, pathways and mechanisms underlying these effects are largely unknown. Casein is the most abundant protein in cow´s milk and it also contains large amounts of BCAAs. However, in contrast to the more soluble whey protein, casein precipitates at low pH and by attack of the enzymes chymosin and pepsin in stomach. This can delay gastric emptying as shown in animal models which in turn could affect incretin secretion and insulin output. Based on human studies on leucine metabolism a model was proposed according to which whey protein is a *"fast"* and casein a *"slow"* digestible protein. Glycomacropeptide (GMP) is a glycosylated polypeptide which is released from casein during the cheese making process and migrates into the whey fraction. It does not contain any aromatic amino acids and moreover, the amino acids arginine, histidine, and cysteine have very low abundance. Effects of GMP on glucose homeostasis and on the metabolic response in humans have never been studied and our studies are thus novel.

The present work aimed to assess whether oral administration of 50g of whey protein isolate as compared to 50g of Na-caseinate or 50g of GMP - each in combination with 50g of a starch hydrolysate (maltodextrin19, MD19) - evoke different effects on glucose homeostasis and the metabolic response in 15 healthy and 15 prediabetic volunteers over a time period of four hours. Since it has been demonstrated that proteins have more pronounced effects in people with disturbed glucose metabolism we not only studied healthy but also well phenotyped prediabetic volunteers. All participants received the three test diets and effects were compared to the administration of MD19. Analysis included the assessment of gastric emptying and oro-cecal transit time and biochemical measurements of blood glucose, plasma GIP, GLP-1, C-peptide, insulin, glucagon, ghrelin, amino acids, acylcarnitines, non-esterified fatty acids and β-hydroxybutyrate, enzyme activities of dipeptidyl peptidase IV, superoxide dismutase, catalase and glutathione levels as well as the feeling of hunger.

Key findings are that whey and caseinate evoked comparable metabolic responses in healthy and prediabetic volunteers. Both proteins elicited a marked reduction in blood glucose concentrations when compared to maltodextrin and this was more pronounced in prediabetic volunteers. The elevation of plasma insulin levels after intake of the milk proteins was not only attributable to a higher insulin secretion rate as indicated by a higher insulin/C-peptide ratio which suggested differences in systemic insulin degradation and removal. No differences in the metabolic responses between the

proteins could be observed by almost identical increases in plasma amino acids. Strong correlations were observed between the content of amino acids in the ingested protein fraction and the postprandial amino acid response and moreover, selected acylcarnitines could be identified as the likely degradation products of the BCAAs. When prediabetic subjects were studied, higher blood glucose and plasma insulin levels were observed – as predicted – but we also found marked differences in plasma amino acid profiles between healthy and prediabetic volunteers. No alterations in any of the measured enzyme activities as part of oxidative defense or in glutathione levels could be observed.

Although the physicochemical properties of GMP are clearly different to that of the other milk proteins, GMP did not produce any significant difference as compared to whey and casein in most of the analyzed parameters. GMP appeared to have a faster gastric emptying compared to casein and whey but induced a similar reduction in blood glucose levels despite a much less pronounced insulin response. GMP also induced the strongest decrease in plasma non-esterified fatty acids and β-hydroxybutyrate despite low systemic insulin levels.

In summary, all three milk proteins in a dose of 50g on background of 50g of maltodextrin produced very similar postprandial metabolic responses in healthy and prediabetic volunteers. That we did not find any differences between whey and casein may be attributed to the use of sodium caseinate rather than a micellar casein as more frequently used. Novel findings within the present work are a) marked differences in postprandial amino acid plasma profiles between healthy and prediabetic volunteers, b) the identification of specific acylcarnitines and other metabolites as degradation products of amino acids entering circulation and c) a marked glucose lowering effect of glycomacropeptide despite its completely different physicochemical properties.

Zusammenfassung

Das Interesse an Nahrungsinhaltsstoffen zur positiven Beeinflussung des Glukosestoffwechsels steigt vor dem Hintergrund der Adipositas- und Diabetes-Problematik. In den letzten Jahren ist dabei dem Molkenprotein der Kuhmilch besondere Aufmerksamkeit zugekommen. Es hat einen hohen Gehalt an verzweigtkettigen Aminosäuren (BCAAs), wobei vor allem dem hohen Leucingehalt eine bedeutende Rolle zugeschrieben wird. Das Molkeprotein vermag die Insulinsekretion direkt zu steigern und auch indirekt über die Sekretion der Inkretinhormone Glukose-abhängiges insulinotrophes Peptid (GIP) und Glukagon-ähnliches Peptid 1 (GLP-1). Allerdings sind die dafür verantwortlichen Mechanismen und involvierten Stoffwechselwege bisher nur unzureichend erforscht. Casein ist das dominante Protein in der Kuhmilch. Auch es weist einen recht hohen Gehalt an BCAAs auf. Im Gegensatz zum Molkeprotein präzipitiert Casein jedoch in Gegenwart von Säure (Magensäure) und den Enzymen Chymosin und Pepsin. Eine in der Folge verzögerte Magenentleerung durch die Koagulation der Caseinmizellen im Magen ist bisher hauptsächlich in Tiermodellen nachgewiesen worden. Dennoch gilt Molkenprotein auch beim Menschen als schnell verdauliches und Casein als langsam verdauliches Protein. Diese Klassifikation beruht vor allem auf Befunden eines unterschiedlichen Leucinmetabolismus bei oraler Verabreichung der beiden Proteine. Aufgrund der unterschiedlichen physikochemischen Eigenschaften und vermeintlichen Unterschiede in der Verdauung wurde auch eine unterschiedliche Beeinflussung des postprandialen Glukosestoffwechsels angenommen.

Glykomakropeptid (GMP) ist ein heterogen glykosyliertes Polypeptid, welches bei der Käseherstellung vom Casein abgespalten wird und in die Molkefraktion übergeht. GMP ist arm an Arginin, Histidin und Cystein und enthält keine aromatischen Aminosäuren. Wirkungen von GMP auf die Glukosehomöostase und den Stoffwechsel im Menschen sind bisher nicht nennenswert untersucht worden. Die dieser Arbeit zugrundeliegenden Humanstudien prüften, ob die orale Gabe von je 50g eines Molkenproteinisolates, Natriumcaseinats oder GMPs bei gleichzeitiger Gabe von 50g eines Stärkehydrolysates (Maltodextrin19, MD19) unterschiedliche Effekte hervorrufen. Dazu wurden Parameter wie Magenentleerung, oro-caecale Transitzeit, Blutglukose, Plasmaspiegel von C-Peptid, Insulin, GIP, GLP-1, Glucagon und Ghrelin, Sättigung, Aminosäurenspiegel, Acylcarnitine, nicht veresterte freie Fettsäuren, β-Hydroxybuterat, Glutathion sowie Enzymaktivitäten von Dipeptidylpeptidase IV, Superoxiddismutase und Katalase bei gesunden Probanden und Prädiabetikern untersucht.

Die erhobenen Befunde belegen, dass es keine nennenswerten Unterschiede in den metabolischen Antworten zwischen den Proteinquellen gibt. Alle führten im Vergleich zur Verabreichung von MD19 zu einer stärkeren Senkung des Blutglukosespiegels, wobei dies in deutlicherer Ausprägung bei

Prädiabetikern zu beobachten war. Die gesteigerte Insulinwirkung war dabei nicht ausschließlich durch eine vermehrte Sekretion erklärbar, da ein erhöhtes Insulin/C-Peptid Verhältnis vorlag. Es ist daher zu vermuten, dass auch die Insulindegradation beeinflusst wurde. Ein nahezu identischer Anstieg der Aminosäurenspiegel im Plasma belegt, dass auch die Magenentleerung und die intestinale Transitzeit sowie die Geschwindigkeit der Verdauung zwischen den Proteinen nicht unterschiedlich waren. Das ist wahrscheinlich darauf zurückzuführen, dass das Natriumcaseinat im Vergleich zum häufiger verwendeten micellaren Casein besser löslich ist. Enge Korrelationen wurden zwischen dem Gehalt der Aminosäuren in den Proteinen und den postprandialen Aminosäureprofilen im Plasma beobachtet. Neuartige Befunde und damit neue Einblicke in den postprandialen Stoffwechsel der Aminosäuren erbrachten die Metabolitanalysen im Plasma mit dem Nachweis der Bildung distinkter Acylcarnitine als Produkte des postprandialen BCAAs-Abbaus sowie der Bildung diverser nicht-proteinogener Aminosäuren aus den Vorstufen.

Auch wenn sich die physikochemischen Eigenschaften von GMP durch die Glykane sowie das Fehlen aromatischer Aminosäuren stark von denen des Molkenproteins und Caseins unterscheiden, konnten nur mariginale Unterschiede in der Stoffwechselantwort beobachtet werden. So führte GMP zu einer schnelleren Magenentleerung und die Senkung des Blutzuckerspiegels ging mit einer deutlich reduzierten Insulinantwort einher. Die stärkste Abnahme der nichtveresterten Fettsäuren und von β-Hydroxybutyrat im Plasma war interessanterweise nach Gabe vom GMP zu beobachten.

Zusammenfassend lässt sich festhalten, dass es keine markanten Unterschiede in der gastrointestinalen Prozessierung der eingesetzten Proteinfraktionen aus der Kuhmilch gibt, wenn diese zusammen mit Kohlenhydraten oral verabreicht werden. Auch die postprandialen Hormonspiegel zeigten sowohl bei gesunden Probanden als auch bei Prädiabetikern keine nennenswerten Unterschiede zwischen den Proteinen – wenngleich alle eine Wirksamkeit auf die Blutzuckerspiegel aufweisen. Der Einsatz von Natriumcaseinat mit der höheren Löslichkeit als mizellares Casein könnte erklären, warum keine Unterschiede zwischen den Proteinquellen vorgefunden wurden. Beobachtete Differenzen in den postprandialen Metabolitkonzentrationen im Plasma hatten ihren Ursprung vor allem im unterschiedlichen Gehalt der Aminosäuren in den Proteinquellen. Neue Befunde wurden zur Bildung von Abbauprodukten diverser Aminosäuren erhoben. Weiterhin ergaben sich signifikante Unterschiede in den postprandialen Veränderungen einiger Plasmaaminosäuren zwischen stoffwechselgesunden Probanden und Prädiabetikern. Bemerkenswert war die Wirkung von Glykomakropeptid, das eine bedeutende Senkung des Blutglukosespiegels bei einer gleichzeitig niedrigeren systemischen Insulinantwort hervorrufen konnte.

1 Introduction

Diabetes has emerged as one of the most challenging health problems of the 21st century. Already described in the Egyptian Ebers Papyrus (King et al. 2003) diabetes remained for 2000 years a non-treatable disastrous and mortal affliction. Aretaeus the Cappodacian (approx. 80-120 A.D.) coined "diabetes" from the Greek word for "siphon" (Leopold 1930) and Thomas Willis, an English physician, added in 1675 the word "mellitus" (DM) to diabetes because of the sweet taste of the urine. Therefore, diabetes mellitus was also called "Willis Disease" (Roy 2008).

The International Diabetes Federation (IDF) defines it as a chronic disease that arises when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Failure to produce insulin, or of insulin to act properly, or both, leads to raised glucose levels in the blood. It belongs to the non-communicable diseases (NCD) comprising also cardiovascular diseases, cancers and chronic lung diseases. In 2004, the mortality of these diseases exceeded by 60% that of infectious diseases, maternal, perinatal and nutritional conditioned disorders (WHO 2008). According to the IDF, the top 10 countries for diabetes cases in 2013 were China (98.4 m), India (65.1 m), USA (24.4 m), Brazil (11.9 m), Russian Federation (10.9 m), Mexico (8.7 m), Indonesia (8.5 m), Germany (7.6 m), Egypt (7.5 m) and Japan (7.2 m). It is predicted to further increase by 55% worldwide till 2035 (IDF 2013). An epidemiological study conducted in centres of Europe, North America and East Asia between 1995 and 1997 found that 25% to 90% of amputations were associated with diabetes (Unwin 2000). Furthermore, diabetic retinopathy was observed to be the 5th leading global cause for blindness in the year 2002 (Resnikoff et al. 2004). In addition, people suffering from diabetes have a more than two-fold higher risk for stroke (Boden-Albala et al. 2008) and a three-fold higher risk for tuberculosis (Jeon et al. 2008). Altogether, about 44% of NCD-related deaths occur before the age of 70 (WHO 2010). The huge increase in diabetes associated diseases leads to a burden for national health care systems worldwide which on average have to spend 12% of their budget for the treatment of diabetes alone (Zhang et al. 2010).

Most NCDs are linked with four avoidable characteristics: physical inactivity, unhealthy nutrition, smoking and alcohol misuse. These characteristics lead to four metabolic outcomes: Overweight and obesity, hyperglycemia, hyperlipidemia and elevated blood pressure (Hu 2011, Haslam & James 2005). Obesity has doubled in men and nearly doubled in women between 1980 and 2008 (Finucane et al. 2011) and data from health examination surveys and epidemiological studies revealed a worldwide increase of diabetes of 18% in men and 22% in women during this time (Goodarz et al. 2011).

The administration of drugs is indispensable when diabetes has progressed. Metformin is a first line drug for type 2 diabetes which acts not on insulin secretion. It reduces insulin resistance, suppresses hepatic glucose production, enhances glucose uptake, increases glucagon-like peptide 1 (GLP-1)

activity, elevates fatty acid oxidation and causes a lower incidence of hypoglycemia (Kim et al. 2008, Collier et al. 2006, Thondam et al. 2012, Davidson et al. 1997). Exenatide, a synthetic form of exendin-4, was approved by the US Food and Drug Administration for the treatment of type 2 diabetes in 2005. Exendin-4 was one of the first incretin mimetics and was found in the saliva of the gila monster (*Heloderma suspectum*) in 1990. It displays a 50% homology to the amino acid sequence of the mammalian incretin hormone glucagon-like-peptide-1 (GLP-1) which promotes insulin secretion. A special feature is its resistance to cleavage by dipeptidyl peptidase-4 (DPP-4) and a longer half-life of 60-90 min as compared to the rapidly degraded human GLP-1 (Drucker et al. 2006, Eng et al. 1990).

Next to the development of anti-diabetic drugs, there is growing interest in nutrition and food research to target diabetes and related metabolic impairments by dietary means. Amongst the macronutrients, proteins were identified as affecting impaired glucose tolerance (Nuttall et al. 1984). Especially for milk proteins a wide spectrum of effects were described and attributed mainly to bioactive peptides formed during digestion and their rather high content of branched chain amino acids (BCAAs). For whey protein, it was also shown that it is also able to increase GIP and GLP-1 levels and in turn promote insulin secretion (Jakubowicz et al. 2013). It has been demonstrated that a whey preload increased insulin secretion by 52% compared to placebo (Jakubowicz et al. 2014) and this reflects the potential of the early insulin increase after a breakfast with 2 mg of repaglinide, which is a short-acting insulin secretagogue (Cozma et al. 2002).

1.1 Effects of protein in human metabolism

Carl von Voit (1831-1908) declared a definite ratio of macronutrients in the diet as important and this was called the "Voitsche Kostmaß". He recommended a daily intake of 118g protein, 56g fat and 500g carbohydrates for a 70kg man working moderately heavy. It is unknown how von Voit came to this recommendation and the calculated ratio of macronutrients (Heyll 2007). Later it was shown that von Voit had overestimated the necessary intake of protein (Hindhede 1922). The current reference values defined by the German, Austrian and Swiss societies for nutrition (DACH) are > 50% of daily energy should come from carbohydrates, 30% from fat and 10% from protein (Deutsche Gesellschaft für Ernährung 2008). However, it has been suggested that intake of protein should be increased as it promotes satiation and increases thermogenesis and thus energy expenditure (Petzke et al. 2007, Westerterp-Plantenga et al. 1999). In obese and insulin-resistant patients, a reduced thermogenesis after food intake has been demonstrated (Tappy 1996) which might reflect diminished energy expenditure. The net metabolizable energy for protein is 13 kJ/g and the thermic effect of nutrients depends on the stimulation of energy-requiring processes which is based on the amount of ATP necessary for metabolism and storage (Westerterp-Plantenga et al. 2006). The thermal effect of dietary protein is between 23% - 30% and therefore much higher than that of carbohydrates (5% - 10%) and that of lipids accounting to only 2% - 3% (Nair et al. 1983). For example, the energy which is necessary for ATP synthesis from protein varies from 99.2 (glutamate) to 153.2 kJ/mol ATP (cysteine), whereas from fat it accounts to 96.3 kJ/mol and from carbohydrates to 91 kJ/mol ATP (van Milgen 2002). Especially, protein synthesis, gluconeogenesis and urea production require high amounts of ATP (van Milgen 2002, Stryer 1988). This need for ATP may be met by a higher fat oxidation rate leading to a negative energy balance (Lejeune et al. 2006, Ditschuneit et al. 1984). However, protein metabolism also depends on the amino acid composition and the digestibility of the protein source (Pannemans et al. 1998).

An elevation of GLP-1 secretion has been shown after high protein intake and that is associated with increased satiety (Lejeune et al. 2006). The satiety effect was proposed to originate in the gut by elevated gluconeogenesis in epithelial cells with sensing of the glucose in portal vagal afferent fibres (Mithieux et al. 2005). High protein diets also stimulate insulin secretion and appear to elevate insulin sensitivity with increased glucose disposal, glucose oxidation and preservation of lean body mass for example after a weight loss. Especially leucine but also arginine, phenylalanine and tyrosine were shown to have insulinotropic activity (van Loon et al. 2000, Piatti et al. 1994) and fasting insulin levels in hyperinsulinemic obese men were reduced to normal range after a high protein diet (Baba et al. 1999). Leucine which is abundant in particular in whey protein, has also been shown to increase muscle protein synthesis through regulation of phosphorylation of translational repressor 4E binding protein1 (4E-BP1) and the ribosomal protein S6 kinase S6K1 (Kimball et al. 2001).

In summary, reported beneficial effects of dietary proteins are attributed to increased satiety, reduced energy efficiency, increased thermogenesis and changes in body composition by promoting lean mass and by improving glycemic control (Skov et al. 1999, Westerterp-Plantenga et al. 2004, Dumesnil et al. 2001, Raben et al. 2003, Yancy et al. 2004, Layman et al. 2003). However, literature also reports negative effects of high protein diets. A high protein diet provided for six months to healthy subjects led to a higher glucose-stimulated insulin secretion, impaired the suppression of hepatic glucose output by insulin and promoted gluconeogenesis (Linn et al. 2000, Rossetti et al. 1989). Furthermore, a promotion of insulin resistance induced by amino acids was also described (Tremblay et al. 2001) emphasizing that effects of an acute or chronic high protein ingestion on glucose homeostasis are still elusive.

1.1.1 Whey protein

Whey proteins are the second dominant protein fraction of bovine milk. Chymosin, an enzyme present in the stomach of calves, leads to coagulation with whey retained in the supernatant. Proposed biofunctional properties of whey have received considerable attention (Smithers 2008). Whey proteins are globular in structure and consist mainly of α-helix motifs with a consistent abundance of acidic/basic and hydrophobic/hydrophilic amino acids. The whey protein fraction consists of several proteins with β-lactoglobulin as the main fraction (< 57%) followed by α-lactalbumin (< 25%), immunoglobulins (< 15%), albumin, lactoferrin (1%) and lactoperoxidase with < 1% (Souza et al. 2012). Whey contains large amounts of the branched chain amino acids (BCAAs) which have been shown to possess insulinotropic potential (Nilsson et al. 2007). In addition, whey protein has a high biological value exceeding that of egg protein as reference protein by 15% (Smithers 2008). In the last decades numerous attempts have been made to generate and fractionate peptides from digest of whey (and other proteins) with a variety of proposed biological activities (Souza et al. 2012, Muro et al. 2011). Whey protein or peptides derived thereof have been shown to affect serum lipids and lipoproteins (Berthold et al. 2011), glycaemia, insulin secretion and action, satiety, weight loss, vascular function and blood pressure (Pal et al. 2010, Hall et al. 2003, Nilsson et al. 2004, Pilvi et al. 2008) as well as muscle protein synthesis (Pennings et al. 2011).

Almost 40 years ago it was shown that intestinal absorption rates of amino acids are more than 3-fold higher when they were administrated as dipeptides compared to the free form (Adibi et al. 1971, 1975). An estimate of the enzymatic products of protein digestion detected in the duodenum and upper jejunum revealed a peptide fraction (50-70%) compared to free amino acids (20-40%) and intact proteins accounting for around 5-10% (Tomé 1987, Baglieri et al. 1995). Only di- and tripeptides but not larger oligopeptides are taken up in intact form into the intestinal cells via the oligopeptide transporter PEPT-1 (Fei et al. 1994). Interestingly, the whey protein β-lactoglobulin and also immunoglobulins were found in intact form in the upper jejunum (Mahé et al. 1991, 1996, Ross et al.

1995) and Chabance et al. also assessed protein degradation from yogurt and milk in humans. They could not find any peptides from whey in the stomach but identified two peptides from bovine lactoferrin in the duodenum 20 min after milk ingestion (Chabance et al. 1998). Notably, the bioactive peptide Ile-Pro-Ala (IPA), released from β-lactoglobulin after hydrolysis revealed a high potential to inhibit dipeptidylpeptidase IV (Tulipano et al. 2011) and thus could reduce the degradation of the incretin hormone GLP-1. Moreover, IPA was shown to have strong antihypertensive effects after oral administration in a rat model (Abubakar et al. 1998). Consistent with the release of peptides from whey digestion, higher levels of GIP and GLP-1 were observed in rodents as well as in humans (Gunnarsson et al. 2006, Frid et al. 2005, Nilsson et al. 2004). There are numerous studies that assessed production and putative functions of whey-derived peptides but mostly with findings *in vitro* and a few animal studies (Morifuji et al. 2009, Pihlanto-Leppälä et al. 1997, Antila et al. 1991). Whether these findings are relevant for the human condition needs to be determined.

1.1.2 Casein

Caseins are insoluble proteins and can be classified into α_{S1}, α_{S2}, β- and κ-caseins. They have a high content of proline and hydrophobic amino acids, lack cysteine and contain also phosphoseryl residues. Glycan structures are only found in κ-casein. Caseins are flexible in structure by lack of SH- and SS-groups. More than 96% of the casein generates colloidal structures in milk which are stabilized by calcium and phosphate. κ-casein is located on the surface of the casein micelle and is thus the prime substrate of chymosin. The cleavage of κ-casein by chymosin or by pepsin in the stomach releases the glycomacropeptide and causes a shift in charge promoting hydrophobic interactions that lead to aggregation and formation of a gel. Moreover, the acidic environment in stomach promotes the precipitation of casein through the removal of calcium (Lebensmittelchemische Gesellschaft 1991, Franzke 1996). Numerous bioactive peptides have been characterized as degradation products of the various casein subfractions with proposed antithrombotic, antihypertensive, immunmodulatory and antimicrobial effects (Mizuno et al. 2005, Parker et al. 1984, Maruyama et al. 1987, Fiat et al. 1993). Some of them were shown to interact with opiod recetors by which they could affect gastrointestinal motility as well as electrolyte and water balance in the gut (Daniel et al. 1990 a, b, Brandsch et al. 1994, Schusdziarra et al. 1983 a, b). Furthermore, antinociceptive and sedative effects were attributed to β-casomorphins which are released after hydrolysis of casein (Brantl et al. 1982, Fiat et al. 1993).

1.1.3 Glycomacropeptide

Glycomacropeptide (GMP), a 64 amino acid peptide, is released by cleavage of κ-casein. It is glycosylated at threonine 131, 133, 135, 142 and serine 141 and cleaved by chymosin at the peptide bound between Phe and Met at position 105/106 revealing the insoluble para- and the polar

glycomacropeptide (Yvon et al 1994). The threonine content of GMP exceeds that of whey protein by 160% and that of casein by 232%. The lack of amino acids like tryptophan, phenylalanine, tyrosine, arginine and histidine and the simultaneous presence of high amounts of BCAAs represent an extraordinary composition. Isoleucine is the most prominent BCAA within GMP. Abbreviations for GMP vary in literature; it is also called caseinmacropeptide (CMP, cGMP), casein-derived peptide (CDP) or caseinglycopeptide (CGP).

During digestion, the glycopeptide has been shown to inhibit gastrin release and gastric acid secretion in the stomach of rats (Fiat et al. 1993, Stan et al. 1982, Stan et al. 1983). Furthermore, caseinmacropeptide has been detected in blood after oral administration in rats suggesting a high proteolytic resistance (Fosset et al. 2002); both, the glycosylation and the high proline content may be responsible for this higher resistance to proteolysis. A study of Chabance et al. reported cCGP in plasma of newborns in biological relevant concentrations although the main part of cCGP is cleaved predominantly in the duodenum probably by the action of trypsin, chymotrypsin and carboxypeptidases (Chabance et al. 1995, Chabance et al. 1998). Boutrou et al. described effective CMP digestion by enzymes of the brush border membrane independent of the glycosylated state of CMP. The digestion of glycosylated and unglycosylated CMP variants by exopeptidases was not different whereas the attached O-glycans obviously restricted the activity of endopeptidases. The degree of glycosylation as well as the number of attached glycans were shown to alter the kinetics of digestion with highly glycosylated species showing slowest digestion (Boutrou et al. 2008). GMP is due to the lack of aromatic amino acids an interesting peptide in the diet of people suffering from phenylketonuria (PKU). A decrease in ghrelin levels and a stronger feeling of satiety in people with PKU was reported after a breakfast with GMP compared to a breakfast including free amino acids only (MacLeod et al. 2010). However, some studies that assessed satiety and cholecystokinin secretion failed to show an effect of GMP (Keogh et al. 2010, Gustafson et al. 2001).

1.1.4 Amino acids involved in glucose metabolism

Glucose is the most potent insulin secretagogue. However, dietary proteins and amino acids derived thereof can also stimulate insulin secretion. An additive effect was observed when carbohydrates were administered together with protein in non-diabetic volunteers and the effect was similar in synergism in type 2 diabetics (Nuttall et al. 1984, Krezowski et al. 1986). Ronner et al. proposed that amino acids stimulate insulin secretion via amino acid sensors in β-cells and a reduced activity of K_{ATP} channels (Ronner et al. 2001). Suggested insulinogenic amino acids are phenylalanine, arginine, lysine, alanine, leucine and isoleucine (Newsholme et al. 2005). Arginine only shows insulinogenic potential when administred intravenously but not when given orally (Dupré et al. 1968) because it is rapid converted into ornithine and citrulline in the intestine (Yu et al. 2003). Among all amino acids, leucine is the most potent substrate to stimulate insulin secretion (Milner et al. 1970). In a physiological dose range

it acts synergistically with glucose and increases insulin secretion by around 70% in humans. This is associated with the production of α-ketoisocaproate and its degradation to acetyl-CoA entering the TCA cycle (Kalogeropoulou et al. 2008, Da Silva et al. 2012). Glutamine (10 mmol/l) and leucine (10 mmol/l) together significantly increase insulin output in perfused rat pancreas whereas glutamine alone can not do this because it is metabolized to γ-aminobutyric acid which affects pancreatic α-cells (Sener et al. 1981, Fernandez-Pascual et al. 2004). However, high glucose concentrations diminish the glutamine flux through glutaminase and GLDH in β-cells (Gao et al. 1999). Alanine and glutamine are also involved in the regulation of β-cell specific gene expression (Newsholme et al. 2005). In contrast to glutamine, glutamate was proposed to stimulate insulin secretion by promoting Ca^{2+}- dependent vesicle exocytosis (Maechler et al. 1999). Alanine (2-10 mmoL) potentiates insulin output by enhancing membrane depolarization followed by an increase in intracellular Ca^{2+} (Dunne et al. 1990). Arginine and leucine as well as arginine and phenylalanine were shown as synergistic combinations to stimulate insulin secretion in man when administred by infusion (Floyd et al. 1970). Interestingly, a strong synergistic effect on insulin secretion by 270% was observed with glycine, proline and hydroxyproline as found in gelatin (25g) applied in humans together with 50g glucose (Gannon et al. 1988). Among the BCAAs, 1 mmol/l isoleucine alone had only a weak effect on insulin secretion but elevated glucose uptake in humans (Nuttall et al. 2008). Moreover, a mixture of 10 essential amino acids has been shown to induce a stronger insulin secretion than any single amino acid in an *in vitro* model (Milner et al. 1970) whereupon most of the amino acids evoke insulin release in combination with others and together with glucose. Acyl-CoA derivatives were next to glutamate also shown to stimulate insulin secretion (Newsholme et al. 2005). Although these findings demonstrate that various amino acids and combinations therefore can cause a release of insulin from ß-cells on background of elevated glucose levels, dietary proteins alone – given to humans – do also cause a significant insulin output comparable to glucose alone but simultaneously stimulate glucagon secretion.

The insulinogenic effect of proteins is not only attributed to a direct stimulation of β-cells but may occur also via the release of incretin hormones like GIP and GLP-1 which promote insulin secretion (Salehi et al. 2012, Nilsson et al. 2007). For example, an intraduonal perfusion of an amino acid mixture as well as the oral administration of alanine, arginine, hydroxyproline, cysteine and lysine was shown to stimulate GIP secretion in humans and mice (Thomas et al. 1976, Flatt et al. 1991). Incubation of pancreatic islets with human serum drawn from subjects after ingestion of whey protein and carbohydrate has shown to cause a more than doubled insulin secretion that in the presence of a GIP-receptor antagonist was decreased by 60% (Salehi et al. 2012). This supports an important contribution of GIP to insulin secretion in the presence of amino acids. Recently it was demonstrated that GLP-1 secretion is stimulated strongest by peptones and by dipeptides involving both, the peptide transporter-1 (PEPT1) and the calcium-sensing receptor in a murine cell culture system (Diakogiannaki et al. 2013). Glucagon output was shown to be stimulated by asparagine, glycine and

phenylalanine whereas no effects were shown for valine, leucine and isoleucine after intravenous infusion in sheeps. The stimulation of glucagon secretion was always accompanied by an increase of insulin secretion (Kuhara et al. 1991). Especially, amino acids which are precursors of pyruvate have been proposed to be more glucagonogenic compared to those entering the TCA cycle via α-ketoglutarate and succinyl CoA (Gannon & Nuttall 2010). Glucagon levels correlate with the amount of metabolized protein and was shown to stimulate gluconeogenesis from amino acids in the liver (Boden et al. 1984) as an important counterregulatory process to prevent a hypoglycemia following protein intake with the effects of protein on insulin secretion.

Leucine is a key regulator of the mTOR signaling pathway which also contributes to the maintenance of β-cell mass *in vivo*. Disturbances in mTOR signaling were shown to elicit a decrease in β-cell size (Xu et al. 2001). Amino acids and insulin act together in this pathway to stimulate phosphorylation of mTOR effectors (**Figure 1**). However, leucine can exert a significant insulin secretion but at the same time impairs glucose uptake into cells (Abumrad et al. 1982) with rapamycin - as mTOR inhibitor - completely reversing this effect (Tremblay et al. 2001). The negative effects of amino acids and especially of leucine on insulin action are associated with an inhibitory phosphorylation of insulin receptor substrate-1 (IRS-1) on serine and/or threonine residues and an impaired activation of PI3-kinase as a key effector that modulates glucose transport, inhibits gluconeogenesis and glycogen storage (Tremblay et al. 2001, Katagiri et al. 1996, Sutherland et al. 1995, Yamamoto-Honda et al. 1995). Moreover, BCAAs could be shown to reduce glucose oxidation by repression of pyruvate dehydrogenase activity resulting from an increase of acetyl-CoA levels and the NADH/NAD^+ ratio (Flakoll et al. 1992, Krebs et al. 2002, Chang et al. 1978). The role of leucine in affecting glucose metabolism is still under debate because studies demonstrated also that leucine and isoleucine improved glucose tolerance in rats. Glucose uptake into skeletal muscle via GLUT4 was enhanced after both, leucine and isoleucine in an *in vitro* model whereas glycogen synthase activity was only enhanced by leucine but this was observed under insulin free conditions (Doi et al. 2003, Nishitani et al. 2005).

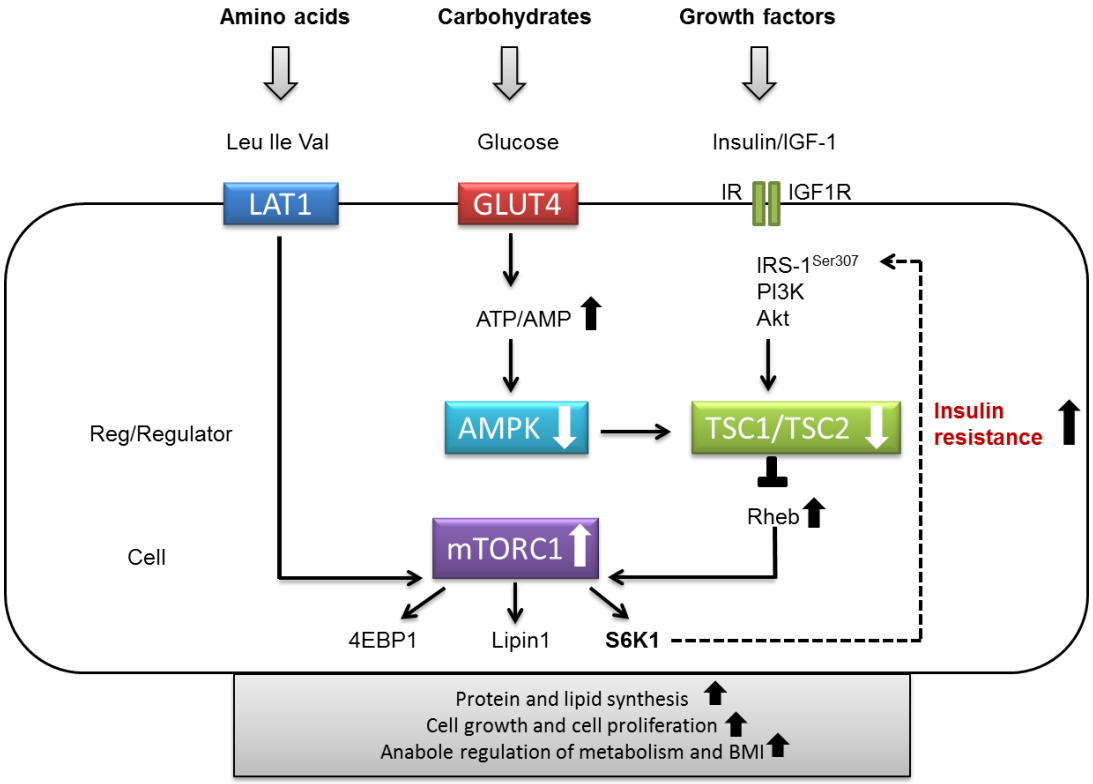


Figure 1: Cross-talk of signalling pathways to affect the mTOR complex 1: Stimulation of mTORC1 by amino acids (valine, leucine and isoleucine), carbohydrates and the growth factors insulin and IGF-1. Activation of S6K1 induces an inhibitory phosphorylation of IRS-1 (insulin receptor substrate 1) followed by insulin resistance (adapted from Melnik 2013).

Abbreviations: LAT1, long neutral amino acid transporter 1, GLUT4, glucose transporter 4, IR, insulin receptor, IGF-1, insulin growth factor 1, IRS-1, insulin receptor substrate 1, PI3K, phosphoinositol 3 phosphate kinase, Akt, proteinkinase B, ATP, adenosine triphosphate, AMPK, adenosine monophosphate kinase, TSC1/2, tuberous sclerosis complex 1/2, mTORC1, mammalian target of rapamycin complex 1, Rheb, ras homolog enriched in brain, 4EBP1, eukaryotic initiation factor 4E binding protein-1, S6K1, S6 kinase 1

1.1.5 The effects of proteins and carbohydrates on satiety

Appetite and food intake are influenced by many exogenous and endogenous factors. Cultural conditioning, environmental availability of food, individual experiences and habitual behaviour belong to exogenous factors. Food intake is regulated by the circadian rhythm, secretion products of adipose tissue, reproductive states and pre- or postabsortive situation. These exogenous and endogenous factors implicate a complex regulation of appetite and satiety (Geary 1990). Alterations in blood glucose concentrations effect glucose sensors in the periphery like in the carotid bodies, in the gastrointestinal tract and the portal mesenteric vein. Glucose sensing neurons are detected in the ventro-medial hypothalamus, arcuate nucleus, paraventricular nucleus, the nucleus tractus solitarii (NTS), area postrema and in the dorsal motor nucleus of vagus in the brain stem (Marty et al. 2007). The hypothalamic arcuate nucleus is a regulatory area processing satiety and adiposity signals. Hunger and satiety are controlled by adjustment of two neuronal circuits that comprise anabolic neurons which produce neuropeptide Y and agouti-related protein and catabolic neurons producing mainly proopiomelanocortin peptides like melanocortin-stimulating-hormone (α-MSH) which can induce a reduction of food intake. High protein diets were shown to activate noradrenergic/adrenergic neurons

which are involved in cholecystokinin-induced satiety (Faipoux et al. 2008). The antagonist kinases mTOR and AMPK (adenosine monophosphate activated protein kinase) are co-localized with neuropeptide Y and pro-opiomelanocortin neurons in the arcuate nucleus and play an important role in hypothalamic energy sensing. AMPK depends on the intracellular ATP/AMP ratio and a high protein diet leads to an inhibition of AMPK and in turn activation of mTOR as an outcome to anorexigenic agents (Davidenko et al. 2013). The availability of certain amino acids like glutamate or leucine in the brain is also suggested to play a role in the central regulation of satiety (Davidenko et al. 2013). A high protein diet was shown to diminish reward-driven eating behavior evoked by a reduced activation of brain areas like the middle prefontal cortex (Leidy et al. 2011).

Russek et al. proposed in 1981 that food intake is mainly dependent on the system that regulates carbohydrate metabolism. According to this model, hunger arises when liver glycogen levels decrease and replenishment by the absorption of glucose and amino acids from the gut may hyperpolarizes the hepatocyte's membrane that in turn could induce satiety via contact to nerve fibres while a second system controls the allocation of energy from the fat stored in adipose tissue which influences the input from the first system (Russek et al. 1981). In contrast to this concept, the "protein leverage hypothesis" from Simpson and Raubenheimer emphasizes that protein and amino acid levels are the most important factors to regulate food intake. This hypothesis addresses especially the ratio of the three macronutrients protein, carbohydrate and fat as more important for energy homeostasis than pure energy intake (Simpson & Raubenheimer 2005). For example, high protein meals could be demonstrated to increase satiety whereas moderate protein intake promotes food intake and diets very low in protein enhance food intake but disturb growth. In the last decades, high protein diets have attracted considerable attention as a mean to tackle obesity because protein is the most potent nutrient in inducing satiety/kcal provided (Westerterp-Plantenga 1999). Clinical studies showed that high protein diets promote weight loss by a decrease in food intake, by maintaining fat-free mass and via an elevation of energy expenditure (Due et al. 2004, Westerterp-Plantenga et al. 2009). A special feature of dietary protein is that it causes cholecystokinin (CCK) secretion and thus increases the firing rate of the vagus nerve transferring information to the nucleus of the solitary tract in brain (Faipoux et al. 2008). Dietary proteins also stimulate GIP and GLP-1 receptors expressed in vagal afferent neurons which act synergistically with CCK and in turn with ghrelin (Moran et al. 2011, Rehfeld et al. 2011, De Lartigue et al. 2011). The ileal break, thought to be mediated mainly by PYY, is also stimulated by proteins inducing proximal gastrointestinal motility and satiety (Maljaars et al. 2008). A high protein diet without carbohydrates causes higher energy expenditure through stimulation of gluconeogenesis and thermogenesis. Proteins deliver less energy (13 kJ/g) compared to carbohydrates (17 kJ/g) and fat (34 kJ/g), which promotes a higher fat oxidation rate and in turn a negative fat-balance. However, a direct effect of eleveated thermogenesis on postprandial satiety is elusive so far (Veldhorst et al. 2009, Westerterp-Plantenga 2008).

1.2 Regulation of gastric emptying

Gastric emptying releases predigested food from the stomach into the gut. This process is controlled by neural and hormonal mechanisms. Solid food displays a biphasic pattern in gastric emptying. At first, it is subdivided and crushed in smaller particles (1-2 mm) and passes the pylorus with a linear emptying rate. Liquids are quickly distributed in the entire stomach. The release of liquids starts immediately and half-emptying of non-caloric liquids is reached after 15-20 min (Meyer et al. 1981). Food with high caloric density leaves the stomach more tardily than low-caloric food which is regulated in a negative feedback loop by duodenal receptors and via CKK (Hunt et al. 1975). This assures a steady-state pattern meaning an unvarying volume of gastric content (Mc Hugh et al. 1983). CKK delays gastric emptying by suppressing antral motility (Fraser et al. 1993). Saline - with no energy - displays an open loop condition with a fast and exponential emptying pattern. Glucose displays a linear emptying rate which is gradually tardier with increasing concentrations (Mc Hugh et al. 1983). It has been proposed that hexoses slow gastric emptying by stimulation of osmoreceptors. Such a sensing mechanism affecting gastric emptying has been demonstrated at a physiological osmolality of 250 mOsmol in the gut. However, different hexose solutions with a fixed osmolarity of 500 mOsmol showed no difference in gastric emptying anymore (Little et al. 2010). Furthermore, in humans with blood glucose concentration of 8 mmol/L when compared to 4 mmol/L, a significantly slower gastric emptying rate was observed similar to that in people suffering from insulin dependent diabetes mellitus (Schvarcz et al. 1997). Studies with glucose have pointed towards a closed loop regulation of gastric emptying on a steady kilocalorie per minute basis which means it is highly regulated compared to gastric emptying of saline which implies an open loop dependent on the volume of gastric contents (Brenner et al. 1983). Furthermore, gastric emptying seems to depend on the length of intestine to which nutritional compounds are exposed. Comparative studies suggested that gastric emptying occurs slower for an acidic chyme as compared to hyperosmolar contents. Gastric content containing fat empties slower than a protein-containing content and this is slower than carbohyrates (Schmidt et al. 2007). The peptide YY and GLP-1 – both secreted from the distal gut – have been shown to reduce motility in the proximal intestine and this was coined "*ileal brake*" (Mortensen et al. 2003). As GLP-1 slows down gastric emptying, it thereby also modulates postprandial glucose profiles (Willms et al. 1996). Gastric emptying rates and transit time are slower by around 35% in women as compared to men and this is attributed to progesterone and estradiol (Caballero-Plasencia et al. 1999, Datz et al. 1987, Schmidt et al. 2007). After finishing digestion, a cyclic order of motor activity, secretion and blood flow moves from the distal stomach towards the ileum which is called the migrating motor complex (MMC) and this is mainly regulated by the enteric nervous system (Vantrappen et al. 1979, Thollander et al. 1996, Thollander et al. 1997).

1.3 The role of peptide hormones in glucose metabolism

1.3.1 Ghrelin

Ghrelin (Growth Hormone Release Inducing) is a growth hormone releasing peptide consisting of 28 amino-acids which is actived by a O-Acyltransferase (GOAT) with a n-octanoyl modification at Ser^3 (Kojima et al. 1999). The absence of the posttranslational acylation of ghrelin appears to affect glucose homeostasis as demonstrated in mice (Zhao et al. 2010). The peptide is cleaved from its precursor preproghrelin. Ghrelin is primarily secreted from X/A cells of the oxyntic mucosa in the stomach with an estimated contribution of around two thirds of the circulating ghrelin. The remaining third originates from X/A-like cells found in the small intestine. The concentration of the acylated ghrelin in blood is 10-20 fmol/ml whereas the total ghrelin concentration is 100-150 fmol/ml in healthy adults. Ghrelin follows an ultradian rhythmicity and shows a half-life in circulation of less than 60 min. It acts as a ligand of the growth hormone secretagogue receptors (GHS-R) which are detected in many tissues and organs (Zhang et al. 2010, Cummings et al. 2001). A blunted nocturnal rise in plasma ghrelin has been demonstrated to be associated with obesity (Bulent et al. 2004). Ghrelin is the only known orexigenic peptide (Bulent et al. 2004). Plasma ghrelin concentrations are elevated during a negative energy balance like during fasting, anorexia, in obese subjects after weight loss and cardiac cachexia. Blood levels are reduced in states of a positive energy balance and in obesity (Tolle et al. 2003, Shiiya et al. 2002, Nagawa et al. 2001). Plasma ghrelin concentration increases before a meal and declines briefly after food intake (Cummings et al. 2001). The intensity of the decline in plasma ghrelin depends on the caloric density and the macronutrient mixture of the meal (Callahan et al. 2004). Ingestion of only protein increases ghrelin concentrations whereas carbohydrate intake alone leads to a reduction (Erdmann et al. 2006). Ghrelin activates food intake by stimulation of Y/agouti gene–related protein neurons (AGRP) and neuropeptide Y in the hypothalamic arcuate nucleus and it reduces fat oxidation to stimulate body weight gain. Ghrelin also influences gastrointestinal motility by which it assumed also to act thereby on appetite signaling. It triggers intense hunger contractions in the fasted state and accelerates gastric emptying (Tack et al. 2005, Tack et al. 2006). A rat study revealed an interaction of bitter taste receptors and α-gustducin in mediating the secretion of ghrelin. This study proposed a direct inhibitory effect of T2R-agonists on gastric contractility (Janssen et al. 2011).

1.3.2 Glucose dependent insulinotropic polypeptide (GIP)

In 1929, Zunz and LaBarre coined the name incretin (INtestine seCRETion INsulin) for hormones secreted from the gut, which influence the secretion of hormones from the pancreas (Zunz et al. 1929). The amplification of insulin secretion after an oral glucose load of 100g by up to eight-fold compared to intravenously administered glucose is called the "*incretin effect"* and is estimated to account for around 40% of insulin output. Incretin hormones are secreted when blood glucose passes a threshold

value of 5.5 - 6 mmol/l (Oya et al. 2013). The incretin hormone GIP was initially defined as an enterogastrone. It is known to inhibit gastric acid release and pepsin output and was therefore initially named ‘gastric inhibitory polypeptide’ (Kosaka et al. 1930, Brown et al. 1969, 1970). Brown et al. isolated GIP and determined the amino acid sequence. The 42 amino acid bioactive form of GIP is derived from its 153-amino acid pro-GIP precursor processed by the proconvertase 1/3 (Ugleholdt et al. 2006) and it is inactivated by the DPP-IV by up to 50% within a few minutes. Dupré et al. showed that infusion of a purified preparation of porcine GIP and glucose elicited a much higher secretion of insulin compared to GIP infusion alone in man (Dupré et al. 1973). The glucose-dependence of GIP action on insulin secretion may be considered as a safety mechanism to protect against hypoglycemia. GIP was the first incretin hormone described and was renamed glucose dependent insulinotropic polypeptide after it was shown to alter insulin output. GIP is predominantly secreted from the proximal intestine from K-cells found in the middle section of duodenal villi with lower K-cell numbers located in the jejunum (Polak et al. 1982). Fat and glucose strongly elicit secretion of GIP (Cleator et al. 1975, Ross et al. 1978, Bestermann et al. 1979) and protein was shown to induce a higher early GIP response compared to fat (Carr et al. 2008). A recent study demonstrated that a dual incretin agonist - acting simultaneously on GIP as well as on GLP-1 receptors – improved adipositas induced insulin resistance and pancreatic insulin deficiency more than individual agonists (Finan et al. 2013).

1.3.3 Glucagon-like-peptide 1 (GLP-1)

Glucagon-like-peptide 1 was discovered by the group of Werner Creutzfeldt in Göttingen in 1985. It is predominantly secreted from enteroendocrine L cells mainly located in the distal ileum, colon and rectum. However GLP-1 is also found in pancreatic alpha-cells and in the caudal brain stem. It derives from proglucagon through post-translational processing by proconvertase 1/3 (PC 1/3) in the intestine (Hansotia et al. 2005, Eissele et al. 1992). GLP-1 has a short half-life of approximately 2 min due to cleavage by dipeptidyl peptidase IV located mainly on endothelial membranes in gut blood vessels (Oya et al. 2013). Further degradation of active GLP-1 takes place in liver and therefore only 10-15% of intact GLP-1 is present in the circulation (Verdich et al. 2001). GLP-1 is found in two bioactive forms, GLP-1 (7-37) and the more available GLP-1 (7-36) amide, both shown to bind to specific G protein coupled receptors (GLP-1R) (Gribble et al. 2003). The concentrations of intact GLP-1 are lower (10-20 pmol/l) than those of GIP which reach up to 100 pmol/l (Vilsboll et al. 2004). Nauck et al. proposed GIP to have a stronger effect on activation of β-cells in man than GLP-1 whereby GIP and GLP-1 when infused together show an additive effect (Nauck et al. 1993). However, a higher potency of GLP-1 to stimulate β-cells was also reported (Elahi et al. 1994). The binding of the incretins to their receptors on β-cells promotes insulin secretion through activation of protein kinase A and the cAMP-regulated guanine nucleotide exchange factor II that causes a closure of K_{ATP} channels and an increase of intracellular calcium concentrations which enhances insulin exocytosis. Moreover,

GLP-1 triggers β-cell proliferation via cAMP response element binding protein (CREB) activation and protects from apoptotic β-cell death in response to hyperglycemia (Drucker et al. 1987, Holz et al. 2004, Sturis et al. 2003). Unger and Eisentraut coined in 1969 the term "*entero-insular axis*" which comprises all endocrine transmissions to the pancreas from the small intestine derived from hormonal, neuronal and direct substrate stimulation of insulin, glucagon, somatostatin and pancreatic polypeptide secretion (Unger et al. 1969, Creutzfeld 1979). The generated products are GLP-1 (9-36) amide and GLP-1 (9-37) which are inactive but were suggested to have antagonist properties at GLP-1 receptors (Deacon et al. 1995). In obese humans, postprandial GLP-1 secretion is decreased compared to normal weight humans (Verdich et al. 2001). Rantes and angiotensin II which are chemokines derived from adipose tissue circulate in higher concentrations in obese humans (Herder et al. 2005) and are suggested to cause inhibition of GLP-1 output. In a mouse model, it could be demonstrated that higher rantes concentrations decreased GLP-1 secretion (Pais et al. 2014). Further, a single nucleotide polymorphism (SPN) in the transcription factor 4 (TCF7L2) was shown to alter intestinal proglucagon expression and this SPN is strongly associated with an increased risk (relative risk 1.35 to 1.56) of type 2 diabetes (Grant et al. 2006). Recent studies showed a strong overlap between proximal L-, K-, and I-cells in the expression levels of GIP, GLP-1 and PYY. Around 10% of L-cells from the proximal intestine were GIP positive and 20% were peptide YY positive, whereas 10% of K-cells in the proximal intestine showed also GLP-1 expression and 6% somatostatin expression (Habib et al. 2012).

It was also demonstrated that the glucose transporter SGLT-1 causes GLP-1 output from endocrine cells in response to luminal glucose and this requires the closure of ATP-sensitive K^+ channels (Gribble et al. 2003). Recently, Oya et al. coud identify a G protein-coupled receptor family (GPRC6A) which induces GLP-1 secretion upon stimulation by amino acids (Oya et al. 2013). GLP-1 is also known to suppress glucagon release from α-cells assumed to happen via stimulation of pancreatic somatostatin release that in turn can inhibit hepatic glucose production in a glucose dependent manner (Nauck et al. 2002, Fehmann et al. 1995). Moreover, recent studies revealed an activation of PC 1/3 in alpha cells in a mouse model of insulin resistance and a switch from glucagon secretion to GLP-1 production (Marchetti et al. 2012, Kilimnik et al. 2010, Liu et al. 2011). GLP-1 is next to PYY, a key mediator in the regulation of the ileal break. GLP-1 delays gastric emptying, regulates gastroduodenal motility and inhibits gastric acid and exocrine pancreatic secretion when nutrients reach the distal ileum. Thus, GLP-1 and PYY, both secreted from L-cells, are proposed to be part of the ileal break via vagal signaling (Schjoldager et al. 1989, Neary et al. 2005, Wettergren et al. 1997, Schirra et al. 2006). Moreover, infusions of GLP-1 in healthy humans increased satiety and reduced food intake (Flint et al. 1998). GLP-1 induces neuroprotective effects through activation of anti-apoptotic signaling pathways in specific neurons and leads to reduced formation of amyloid beta peptide levels in brain (Perry et al. 2003). An overview about the multiple actions of GLP-1 is presented in **Figure 2**.

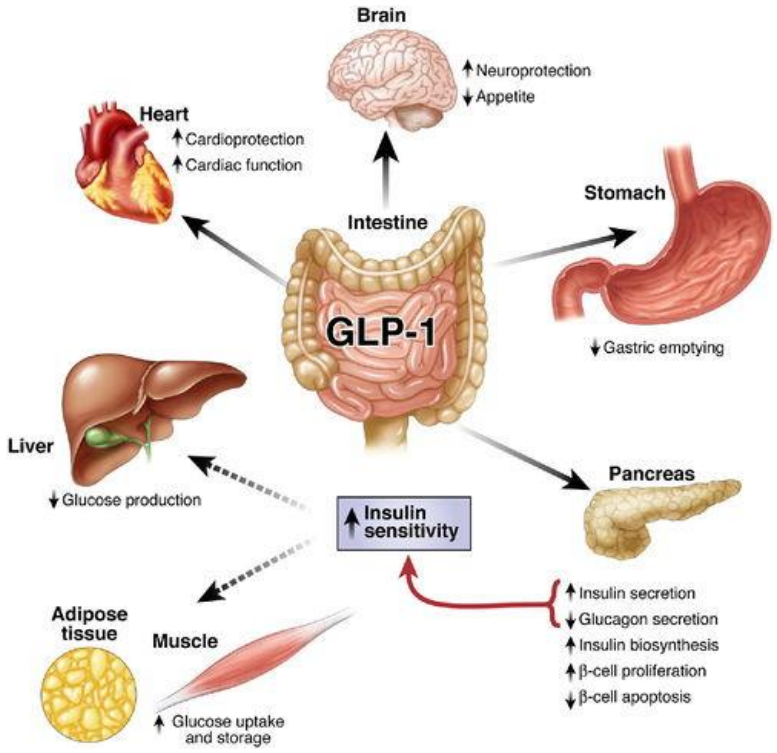


Figure 2: GLP-1 actions in peripheral tissues (adapted from Baggio et al. 2007)

1.3.4 Glucagon

Glucagon, a single-chain polypeptide composed of 29 amino acids, is secreted by pancreatic α-cells and possesses a half-life of 10-15 min. It is the most important hyperglycaemic hormone in the body (Foá et al. 1973, Cryer et al. 2002). Although insulin does not act directly on glucagon secretion, glucagon was shown to stimulate the release of insulin *in vitro* and *in vivo*. Insulin counteracts the hepatic glucagon activity by inducing cAMP phosphodiesterases. Therefore, the ratio of glucagon to insulin is an important factor to determine net glucagon activity (Mc Carty et al. 1999). A stimulation of glucagon output is induced by epinephrine, cortisol, growth hormones, pancreozymin and secretin whereby amylin and GLP-1 inhibit glucagon secretion. Glucagonotrophic effects are also known for gastrin, substance P, neurotensin, bombesin, CCK and vasoactive intestinal polypeptide (Pek & Spangler 1983, Mc Carty 1999). In contrast to GLP-1, GIP does not inhibit glucagon secretion (Yip et al. 2000). Glucagon increases blood glucose concentration via glycogenolysis and gluconeogenesis in liver and promotes ketogenesis as well. During fasting of 8-12 hours, glycogenolysis is the predominant pathway supplying glucose to maintain blood glucose levels before gluconeogenesis takes over (Aronoff et al. 2004). An important step for the action of glucagon after binding to its membrane receptors is the conversion of ATP to cyclic 3,5-adenosine monophosphate by adenyl-cyclase which activates several protein kinases catalyzing the phosphorylation and regulation of the activity of various enzymes involved in the gluconeogenic chain. Substrates for gluconeogenesis are glucogenic amino acids as well as lactate and glycerol. Amino acids that enter the gluconeogenetic

pathway in the form of pyruvate where shown to promote gluconeogenesis more than amino acids entering gluconeogenesis via succinyl CoA or α-ketoglutarate. Essential amino acids stimulate protein synthesis and storage after insulin release whereas non-essential amino acids and arginine promote glucagon secretion (Mc Carty 1999). While glucagon elevates blood glucose and fatty acid levels in serum, it reduces amino acid concentrations indicating a feedback regulation of its own secretion (Foà et al. 1973). Elevated hepatic glucagon leads to a reduction in de novo lipogenesis and in turn to reduced fat storage and cholesterol synthesis, lower circulating LDL cholesterol as well as increased hepatic lipid oxidation due to lower malonyl-CoA levels and a decrease in IGF-1 (Mc Carty et al. 1999). Glucagon refered to as a "gut-brain-peptide" initiates a signal of satiety (Geary 1992). Glucagon also elicits paracrine effects which include stimulation of somatostatin, catecholamines and growth hormone secretion (Patton et al. 1977, Weir et al. 1981, Lefebvre et al. 1983, Merimee et al. 1983). Furthermore, it inhibits pentagastrin-stimulated gastric acid secretion and cholecystokinin-stimulated gall bladder contraction (Diament et al. 1983, Jorgensen et al. 1983). Walker et al. proposed a model for secretion of glucagon including paracrine effects and an intrinsic regulation of glucagon secretion. According to this model, hypoglycemia and physical activity lead to a small opening of the K_{ATP}-channels followed by membrane depolarization whereas an elevation in glucose levels impedes glucagon secretion by closure of the K_{ATP}-channels. The membrane depolarization exerts a decreased amplitude of the α-cell action potential and diminishes the opening of voltage-gated P/Q-type Ca^{2+}-channels which in turn suppresses exocytosis of glucagon-containing vesicles. After a mixed meal, glucagon secretion is abolished by a combination of K_{ATP}-channel closure and activation of paracrine inhibitory signaling due to coincident stimulation of hormone secretion in the neighbouring ß- and δ-cells. Somatostatin could be shown to supersede the stimulatory effect of amino acids and free fatty acids on glucagon release. When free fatty acids and amino acids are increased by mobilization from body depots, a low plasma glucose level leads to an opening of K_{ATP}-channel, membrane repolarization and diminished hormone secretion from β- and δ-cells. Therefore, the absence of inhibitory paracrine signals derived from these cells amplifies the stimulatory effect of low glucose levels in the presence of amino acids and free fatty acids leading to strong stimulation of glucagon output (Walker et al. 2011).

1.4 Insulin resistance

Insulin resistance (IR) as a physiological process occurs during puberty, pregnancy and with ageing (Moran et al. 1999, Buchanan et al. 1990, De Fronzo et al 1979) whereas increased physical activity for example can increase insulin sensitivity (Chen et al. 1988). Type 2 diabetes is a consequence of disturbed pancreatic β-cell function with a final failure to secrete enough insulin to meet the metabolic demand. It is proposed that between 60% and 90% of type 2 diabetes cases originate from an obesity background that is associated with insulin resistance (Anderson et al. 2003). In this respect, adipocytes and adipose tissue are important mediators in the pathogenesis of insulin resistance. Adipokines like resistin, leptin, TNF-α, adiponectin, retinol-binding protein 4 (RBP4) and monocyte chemoattractant

protein-1 (MCP-1) have been identified as pathogenic factors in insulin resistance (Kasuga et al. 2006). Plasma non esterified fatty acids (NEFAs) are increased in obesity and type 2 diabetes and an acute increase of NEFAs leads immediately to insulin resistance in humans (Polonsky et al. 1988) by competition with glucose for substrate oxidation (Randle et al. 1963). Furthermore, glucose metabolism was proposed to elevate mitochondrial production of ROS which could promote an inflammatory status (Lin et al. 2005). This in turn was suggested to cause endoplasmatic reticulum stress with activation of c-Jun N-terminal kinases (JNK) that are known to contribute to insulin resistance (Ozcan et al. 2004). In mice lacking specifically the insulin receptors in liver, insulin resistance, glucose intolerance and a failure of insulin to suppress hepatic glucose production were reported (Michael et al. 2000). In contrast, mice lacking insulin receptors only in muscle revealed a hyperlipidemia but showed normal glucose tolerance (Brunning et al. 1998). Transgenic mice with a negative dominant mutant IGF-1 receptor in muscle also develop insulin resistance (Fernandez et al. 2006). A lack of insulin receptors in adipocytes is associated with a lean phenotype and shows a protection against obesity-related glucose intolerance (Bluher et al. 2002). In the brain, the action of insulin is important for the maintenance of energy homeostasis (Okamoto et al. 2004). Insulin regulates via the brain the suppression of hepatic glucose production (Obici et al. 2002) suggesting that insulin resistance in the brain contributes to the pathogenesis of type 2 diabetes. Deleting insulin receptors in β-cells reveals disturbance in glucose sensing and a reduced β cell mass (Kulkarni et al. 1999). Mice with deficient insulin and IGF-1 receptor signalling develop early-onset diabetes as a result of reduced β-cell mass (Ueki et al. 2006). Loss of β cells is thought to originate from higher exposure of β cells to glucose (glucose toxicity) and/or lipids (lipotoxicity). One hypothesis proposes an altered expression and function of the mitochondrial inner membrane uncoupling protein-2 (UCP2) as a major contributor (Zhang et al. 2001). UCP2 when activated acts as a proton channel across the inner mitochondrial membrane which can uncouple glucose oxidation and ATP production. This leads to a negative glucose-stimulated regulation of insulin production (Chan et al. 2001). Stimulation of UCP2 activity by superoxide leads to a large induction of a proton leak followed by β cell dysfunction (Echtay et al. 2002). Systemic insulin resistance is proposed to be also affected by factors such as Angiopoietin-like 13 (Angptl3) and myostatin as secreted by several cell types (Koishi et al. 2002, Zimmers et al. 2002). Intriguingly, a recent paper demonstrated that a bypass of the duodenum-jejunum elevates insulin sensitivity and insulin clearance but not the appearance of glucose which argues for endocrine factors derived from the gut that may elicit insulin resistance (Salinari et al. 2013). Regarding lipotoxicity and insulin resistance, also fatty acid transporter protein 1 (FATP1) and fatty acid-binding proteins (FABPs) were proposed to contribute to these disturbances (Kim et al. 2004, Maeda et al. 2005). Numerous other factors have been shown to contribute to or promote insulin resistance and type 2 diabetes including brown adipocytes and more recently also the gut microbiota (Caricilli et al. 2013).

2 Objective of the thesis

The objective of this thesis was to investigate the effects of the two main milk proteins casein and whey protein and the casein derived whey peptide glycomacropeptide on the metabolic responses in 15 Caucasian healthy male and 15 Caucasian prediabetic volunteers. The effects of the milk proteins were especially of interest in prediabetic people - not using any oral hypoglycemic drugs - because it has been demonstrated that proteins have more pronounced effects in people with disturbed glucose metabolism. The experimental research focused on the effects on gastric emptying, oro-cecal transit time, glucose homeostasis, hormones involved in glucose metabolism, the feeling of hunger, plasma amino acids, acylcarnitines, activities of enzymes involved in oxidative stress after administration of the milk components in the presence of starch over a time period of four hours.

3 Materials and Methods

3.1 Equipment and Kits

Equipment, kits and chemicals used for the analysis of the samples are illustrated in **Table 1**, **Table 2** and **Table 3**.

Table 1: Equipment and Instruments

Apparatus	Company
Automatic burettes	Brand, Wertheim, Germany
Biofuge 15R	Heraeus Instruments, Hanau, Germany
Bioplex System	Biorad, München, Germany
Centrifuge 5417R	Eppendorf, Hamburg, Germany
Centrifuge 5702R	Eppendorf , Hamburg, Germany
Compartment dryer	Binder, Tuttlingen, Germany
Deltatrac metabolic monitor	Datex-Ohmeda, Helsinki, Finland
Free Style Navigator	Abbott, Wiesbaden, Germany
Filterplate multi Screen Solvinert	Merck-Millipore, Schwalbach, Germany
Galaxy 14D	VWR, Darmstadt, Germany
Gastrolyzer	bedfont scientific Ltd., Maidstone, England
Glucose 201+ Analyzer full-blood calibrated	HemoCue, Großostheim, Germany
LC-MS/MS AB Sciex 3200 Q Trap, 5500 Q Trap	Agilent Technologies, Böblingen, Germany
LC-MS Water Baker J.T.	Avantor, Center Valley, USA
Monovette, neutral	Sarstedt, Nürmbrecht, Germany
Monovette (EDTA coated)	Sarstedt, Nümbrecht, Germany
Multiquick 5 Type 4191	Braun, Kronberg, Germany
New Classic MS	Mettler Toledo, Gießen, Gernany
Osmometer 806	Vogel, Gießen, Germany
Rotina 420R	Hettich , Tuttlingen, Germany
Saftey IV Catheter with injection part 18G+20G	Braun, Kronberg, Germany
Scales	Seca, Hamburg, Germany
SPD Speed Vac	Thermo savant, München, Germany
Stylet 18G + 20G	Braun, Kronberg, Germany
Super GL easy+	Dr. Müller Geräte Bau, Freital, Germany
Thermomixer compact	Eppendorf, Hamburg, Germany
Ultrospec 3100 pro	Amersham, München, Germany
Universal shaker	Bühler, Hechingen, Germany
Urine beaker	Dr. Junghans Medical, Bad Lausick,Germany
UVIKOM 930 Spectrophotometer	Goebel, Au, Germany
Vacutainer	Becton Dickinson, Heidelberg Germany
Varioskan	Thermo Elektron Corporation,München, Germany
Whatman paper	Sigma Aldrich, Taufkirchen, Germany
96 deep well plate, round, bottom plate	Nalge Nunc, New York, USA

Table 2: Kits

Kit	Company
aTRAQ Reagent Kit	ABSciex, Foster City, USA
Bio-Plex Pro diabetes assays	Bio-Rad, München, Germany
D-3-Hydroxybutyric acid	Roche, Unterhaching, Germany
ELISA GLP-1 High Sensitivity Chemiluminescent	Merck Millipore, Schwalbach, Germany
iTRAQReagent Kit (AA45/32^{TM} Phys)	Applied Biosystems, USA
Milliplex MAP Kit Human Metabolic Hormone	Merck Millipore, Schwalbach, Germany
Milliplex MAP Kit Human Serum Adipokine	Merck Millipore, Schwalbach, Germany
NEFA-HR(2)	Wako, Neuss, Germany
Neonatal Standard (Acylcarnitines)	Chromsystems, München, Germany

Table 3: Chemicals and test meal ingredients

Chemicals	Company
Aprotinin	AppliChem, Darmstadt, Germany
Acetaminophen-D4	LGC Standards, Wiesel, Germany
DPIV-Inhibitor	Merck Millipore, Schwalbach, Germany
Glycomacropetide	Davisco, Eden Prairie, USA
Gly-Pro-pNA	Bachem, Bubendorf, Swizerland
HCl	Roth, Karlsruhe, Germany
Hydroxyproline	Sigma-Aldrich, Steinheim, Germany
Lactulose	Hemopharm, Bad Homburg, Germany
Lemon flavour	Dr. Oetker, Bielefeld, Germany
Maltodextrin19	Berco Arzneimittel, Kleve, Germany
Methanol	Merck, Darmstadt, Germany
Na-Caseinate FN 5 S	Rovita, Engelsberg, Germany
Paracetamol (Acetaminophen)	Ratiopharm, Ulm, Germany
PBS	Sigma-Aldrich, Steinheim, Germany
Pepsin	Sigma-Aldrich, Steinheim, Germany
p-Nitroaniline	Sigma-Aldrich, Steinheim, Germany
Tris	Sigma-Aldrich, Steinheim, Germany
Vanilla flavor	Dr. Oetker, Bielefeld, Germany
Whey protein isolate (BiPro)	Davisco, Eden Prairie, USA

3.2 Study design and study methods

The human study was a single-center, single-blind randomized 4-treatment trial (MIPROMET) carried out at the ZIEL Research Center for Nutrition and Food Science of the Technischen Universität München in Freising-Weihenstephan. The study was approved and reviewed by the Ethics Committee of the Technischen Universität München (reference 2436/09) and registered at the Deutsches Register für Klinische Studien (#DRKS00005682). An overwiev of the analysis of the human study is provided on the next page in **Figure 3**.

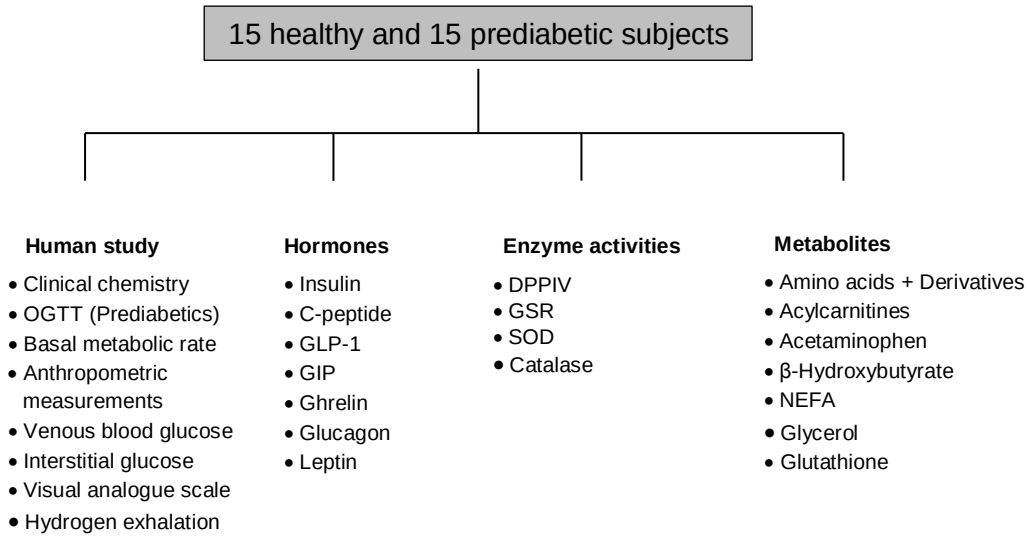


Figure 3: Overview about the analysis in the MIPROMET-study

15 healthy male volunteers aged 21 to 31 years with a BMI between 21.4 kg/m^2 and 27 kg/m^2 and 10 prediabetic male and 5 female postmenopausal subjects aged 51 to 72 years with a BMI between 21.1 kg/m^2 and 41 kg/m^2 underwent the same trial. Participation criteria are illustrated in **Table 4** and an overview about the characteristics of the subject is provided in **Table 5**. Each volunteer ingested a drink consisting of 50g whey protein isolate or 50g sodium caseinate or 50g glycomacropeptide always in combination with 50g maltodextrin19 (starch hydrolysate) and one drink only containing 50g MD19 on four separate days (exact ingredients see **Table 6, Table 7** and **Figure 6**).

Table 4: Participation criteria of subjects in the MIPROMET study

	Healthy group	**Prediabetic group**
Inclusion criteria:	• Healthy young men • Age: 20-35 years • BMI: 18-27 kg/m^2 • Caucasian	• Prediabetic men and post-menopausal women • Age: 50-75 years • BMI: 18-41 kg/m^2 • Prediabetes according to WHO definition (OGTT: fasting plasma glucose level between 110-125mg/dl, 2h glucose 140-200mg/dl) • Caucasian
Exclusion criteria for both groups:	• Chronic diseases • Gilbert's disease • Lactose intolerance • Smoking • Intake of oral hypoglycemic agents • Intake of antibiotics within 6 weeks before the test days • Mental health problem • Excessive physical activity (more than 6 hours per week)	

Individuals were instructed to avoid eating fibres, food with flatulent properties and high fat meals the day before. Furthermore, they were requested to avoid drinking alcohol and undertaking extreme physical activity two days prior the test day. The test days were separated by two days in between.

Seven subjects out of the healthy group and all out of the prediabetic group were equipped with a glucose system allowing continuous measurement of interstitial glucose every 10 minutes.

At 8:00 am, after a 12 h overnight fast, an indwelling catheter was inserted into an antecubital vein of the subjects for blood sampling. After 15 minutes, a baseline blood sample was drawn. The test drink was administered subsequently and consumed within 10 minutes approximately at the same rate in the same volunteer on each occasion. The time schedule of the test days is illustrated in **Figure 4**.

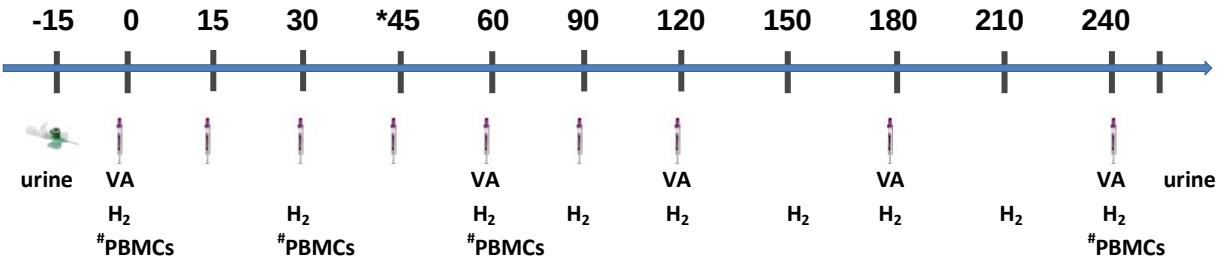


Figure 4: Time schedule of the MIPROMET study in minutes for all four test days

Abbreviations: VA: Visual analogue scale (Assessment of the hunger feeling), H_2: Hydrogen of the exhalation air (Transit time). *Time point 45 was additionally inserted in the study design for all challenges in the prediabetic group. #PBMCs were collected at 0 min and 30 min in the healthy group and at 0 min, 30 min and 240 min in the prediabetic group.

3.3 Recruitment of subjects

Subjects were recruited by public advertisement in local newspapers and bulletins. Additionally, for the recruitment of potential prediabetic volunteers, general practitioners were contacted by personal attendances and by letters.

3.4 Screening

Volunteers were invited to the human study center in the ZIEL research building in Weihenstephan-Freising for the initial health assessment. The volunteers were instructed of the study goals and methods and agreed to participate in the human study by signing the study consent form. They were aware of having the right to leave the study at any time. They were all requested to be fasted 12 hours before the screening day. The health assessment included family anamnesis, clinical chemistry (analyzed by a medical validated lab in Munich), anthropometric measurements, waist/hip ratio and basal metabolic rate, both in the healthy and prediabetic group. Screening data of the participant volunteers are shown in **Table 5**. Furthermore, an oral glucose tolerance test was applied to confirm a prediabetes status. Itemized parameters of the screening data are presented in the appendix (**Table 17**, **Table 18**).

Table 5: Characteristics of the subjects

Subjects	Number	Sex	Age	BMI	Waist	OGTT* 0 min	OGTT* 120 min	HOMA#
			y	kg/m^2	cm	mg/dl	mg/dl	
Healthy	15	m	26±0.60	23.9±0.50	86.6±1.50	-	-	0.64±2.48
Prediabetics	10	m	60±1.53	28.5±1.60	105±4.08	111±2.92	159±7.85	1.99±2.28
Prediabetics	5	f	66±1.56	30.1±1.44	112±2.40	107±2.11	138±4.64	1.88±0.44
All Prediabetics	15	m	62±1.68	29.0±1.52	104±3.97	110±2.64	152±7.29	1.93±0.53

***** An OGTT was only performed with potential prediabetic people

[#] The homeostatic model assessment was calculated from the challenge with 50g MD19

3.5 Oral glucose tolerance test (OGTT)

An oral glucose tolerance test was arranged with assumed prediabetic volunteers. The volunteers were briefed to include 150g carbohydrates in their daily meal during 3 days before the OGTT. Additionally they were requested to maintain a 12 h fasting period overnight. The OGTT was always initiated between 8 and 8.30 a.m. The OGTT consisted of 75g maltodextrin19 (MD19) dissolved in 300 ml water. An indwelling catheter was inserted into an antecubital arm vein. Blood samples were collected with monovettes containing sodium fluoride before drinking (fasting plasma glucose), 1 h and 2 h after beginning of drinking the MD19 solution. Blood samples were sent to a validated medical lab in Munich to analyze venous plasma glucose concentration.

3.6 Basal metabolic rate and respiratory quotient

Basal metabolic rate and respiratory quotient were analyzed by indirect calorimetry using the Deltatrac metabolic monitor (Datex-Ohmeda, Helsinki, Finland). Fasted individuals rested 30 minutes in a supine position covered with a plastic hood over their head. The determined values of the basal metabolic rate and respiratory quotient were noted after 10 minutes and then every 5 minutes. A mean of all measured values was calculated.

3.7 Continuous glucose measurement

The Freestyle Navigator (Abbott, Wiesbaden, Germany) allows to continuously measuring interstitial glucose every minute. The system consists of a sender which can be fixed at the upper arm or at the belly. The receiver can be carried in a pocket. Data transfer is managed wireless within 3 meters. The system generates a mean of values over 10 minutes. Subjects were equipped at the upper arm with the sender and the receiver one day before the test day. The system requires three calibrations with plasma glucose received from arterial blood out of the finger pulp. Subjects calibrated the system after 1 h, 2 h, 10 h, 24 h and 96 h after fixing the equipment. The Freestyle Navigator can be carried for five days.

3.8 Challenges

The test drinks were always prepared freshly in the morning of the test day and the composition is illustrated in **Table 6.** Either 50g whey protein isolate (BiPro) without GMP (Davisco, Eden Prairie, USA) or Na-caseinate (FN 5 S, Rovita, Engelsberg, Germany) or 50g glycomacropeptide (GMP, 30 kDa, 64 AS, 8.5% glycosylated with sialic acid, Davisco, Eden Prairie, USA), 50g maltodextrin19 (MD19, Berco Arzneimittel, Kleve, Germany), 10g lactulose (Hemopharm, Bad Homburg, Germany), 1g acetaminophen (Paracetamol, ratiopharm, Ulm, Germany) and 10 drops of lemon or vanilla flavor (Dr. Oetker, Bielefeld, Germany) were mixed with a hand blender (Multiqick 5, Braun, Kronberg, Germany) in 300 ml water adjusted to room temperature. The amino acid composition of the whey protein isolate, Na-caseinate and glycomacropeptide are shown in **Figure 5**, **Figure 6** and **Table 7**.

Table 6: Composition of the test drinks

Control drink	WPI drink	CP drink	GMP drink
-	50g Whey protein isolate	50g Na-caseinate	50g GMP
50g MD19	50g MD19	50g MD19	50g MD19
10g Lactulose	10g Lactulose	10g Lactulose	10g Lactulose
2g Hydroxyproline[1]	2g Hydroxyproline[1]	2g Hydroxyproline[1]	-
1g Acetaminophen[2]	1g Acetaminophen[2]	1g Acetaminophen[2]	1g Acetaminophen[2]
300 ml water	300 ml water	300 ml water	300 ml water
364 mosm/kg	438 mosm/kg	559 mosm/kg	822 mosm/kg
194 kcal	380 kcal	381 kcal	374 kcal
pH: 7.45	pH: 6.80	pH: 6.49	pH: 6.20

[1] Hydroxyproline was applied in the healthy group

[2]Acetaminophen was applied in the healthy group with the GMP challenge and in the prediabetic group for all challenges

The control drink consisted of 50g MD19, 10g lactulose, 1g acetaminophen and lemon or vanilla flavor dissolved in 300ml room tempered water. The control drink, as well as the WPI and CP drink of the healthy group contained 2g hydroxyproline (Sigma-Aldrich, Steinheim, Germany) instead of 1g acetaminophen.

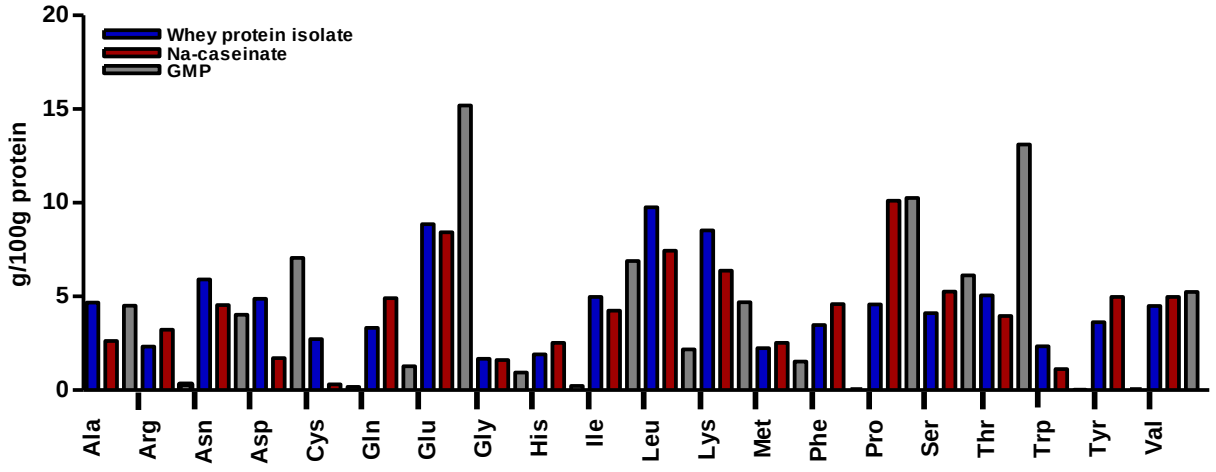


Figure 5: Amino acid spectrum of the milk derived fractions

Hydroxyproline was assumed to be a good marker of the kinetics and velocity of amino acid absorption because it was known from previous studies that there is no change of hydroxyproline in plasma after an OGTT. We exchanged hydroxyproline by acetaminophen in the GMP challenge in the healthy group and in all test drinks in the prediabetic group as a more common noninvasive marker for gastric emptying.

Table 7: Amino acid composition of the milk fractions (g/100g protein)

AA	Whey	Na-Caseinate	GMP
Ala	4.68	2.63	4.51
Arg	2.32	3.22	**0.31**
Asn	5.90	4.54	4.03
Asp	4.87	1.71	7.06
Cys	**2.73**	0.31	0.17
Gln	3.32	4.91	1.27
Glu	8.86	8.43	**15.2**
Gly	1.67	1.61	0.94
His	1.91	2.53	**0.22**
Ile	4.98	4.24	6.89
Leu	**9.75**	7.44	**2.18**
Lys	8.52	6.37	4.70
Met	2.24	2.53	1.52
Phe	3.48	4.6	**0.00**
Pro	4.58	**10.1**	**10.25**
Ser	4.11	5.25	6.13
Thr	5.05	3.95	**13.1**
Trp	2.34	1.12	**0.03**
Tyr	3.62	4.97	**0.00**
Val	4.49	4.97	5.24

The amino acids of the proteins and GMP were analyzed by the Chair of Proteomics and Bioanalytics of TUM, Freising, Germany and Technische Universität Dresden, Chair of Food Chemistry, Germany

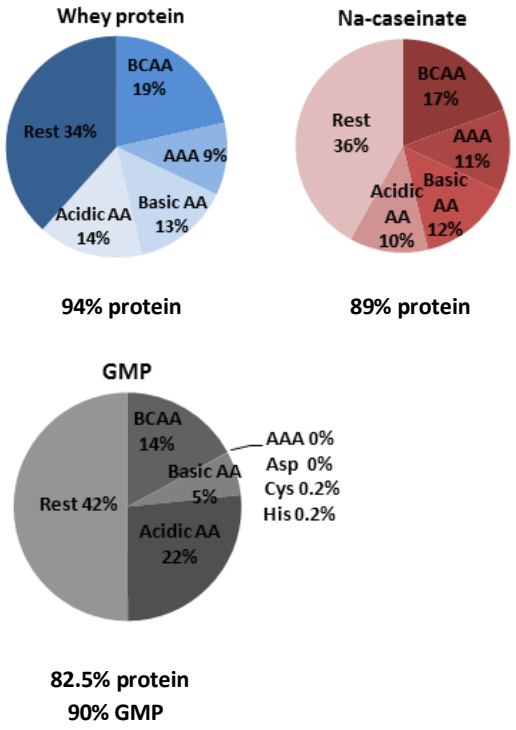


Figure 6: Distribution of amino acids in whey protein isolate, Na-caseinate and GMP

3.9 Sampling

For hormone analysis 10 µl/ml of cold 2.5 mM DPIV-Inhibitor (Millipore, Schwalbach, Germany) and 1.3% of 5,500 KIU/mg of cold aprotinin (AppliChem, Darmstadt, Germany) were prepared in a monovette coated with EDTA (Sarstedt, Nümbrecht,Germany) shortly before blood withdrawal. Blood for isolation of peripheral blood mononuclear cells (PBMC) was drawn at time points 0 and 30 min in the healthy group, and at 0 min, 30 min and 240 min in the prediabetic group, and PBMCs were isolated immediately. For all other analyses, EDTA coated monovettes were inverted gently 10 times and centrifuged at 20°C at 3,000 rpm for 10 minutes. Before centrifugation, venous blood glucose was measured using the Super GL easy^{+} (Glucose oxidase system, full-blood calibrated, Dr. Müller Geräte Bau, Freital, Germany) and the Glucose 201+Analyzer (Glucose dehydrogenase system, HemoCue,

full-blood calibrated, Großostheim, Germany), respectively. After centrifugation, plasma was immediately aliquoted on ice, frozen on dry ice and later stored in a -80°C freezer. Urine was collected before (fasting condition) and after the testing phase, aliquoted and stored at -80°C.

3.10 PBMC Isolation

Blood for PBMC isolation was collected in a neutral monovette (Sarstedt, Nümbrecht, Germany) to avoid hemolysis. Afterwards, blood was transfered in a BD Vacutainer (Becton, Dickinson, Heidelberg, Germany) and centrifugated in a horizontal rotor (swing-out head, Hettich Rotina 420R, Tuttlingen, Germany) at 1750g for 15 min. The white cloud containing the PBMC were collected with a plastic Pasteur pipette and transferred to a falcon tube. PBMC were washed with 13 ml PBS and centrifuged for 15 min at 450g. PBMC were washed again with 2 ml PBS and centrifuged at 1900g for 1 min. Pellets were immediately frozen in liquid nitrogen and stored in a -80°C freezer.

3.11 Assessment of the feeling of hunger

For assessment of subjects' hunger feeling during the test day a visual analogue scale was used (**Figure 7**). This is a psychometric response scale and allows the measurement of subjective characteristics which are not directly possible to measure. Subjects were requested to rate their feeling of hunger by marking the scale with a vertical line on a continuous horizontal line of exact 10 cm width on a sheet of paper.

Figure 7: Visual analogue scale

3.12 Oro-cecal transit time

Test drinks were supplemented with non-digestible lactulose for the assessment of the oro-cecal transit time. Fermentation of lactulose by the intestinal flora delivers H_2 which can be detected in a non-invasive manner in the exhaled breath. An increase in hydrogen in the exhalation air is an indicator for the transit time. The transit time is assessed by calculation of the inflexion point of the hydrogen curve. Hydrogen in the exhaled air was measured by the Gastrolyzer (bedfont, Maidstone, England) every 30 minutes during the test phase. Subjects were asked to inhale deeply and hold their breath for 15 sec. At the end of the countdown, the subjects blew slowly into a mouthpiece with the aim to empty the lungs completely.

3.13 Enzymatic colorimetric tests

3.13.1 D-3-Hydroxybutyric acid

For the analysis of D-3-Hydroxybutyric acid a kit from Roche (Unterhaching, Germany) was applied according to the manufacturer's protocol.

3.13.2 Non-esterified fatty acids

Non-esterified fatty acids were quantified using a kit from Wako following the manufacturer's protocol (Neuss, Germany).

3.13.3 Dipeptidylpeptidase IV

Plasma dipeptidyl peptidase IV (DPPIV) was measured from prediabetic subjects according to the protocol from Sigma Aldrich, Steinheim, Germany. DPPIV cleaves Gly-Pro-p-Nitroanilide into Gly-Pro and p-Nitroanilin (pNA). A standard curve within a range of 1 to 40 nmol was generated with pNA. 50µl plasma and 50µl 0.1M Tris (pH 8.0, 37°C) were pipetted in a microplate. 0.1ml of 1mM Gly-Pro-pNA solution was added to start the reaction. Afterwards the microplate was incubated for 15 minutes. Intensity of the generated color was detected spectrophotometrically at 405 nm. A blank of 0.1 mM Tris and 0.1 ml 1mM Gly-Pro-pNA was substracted from all measurements. A standard curve was plotted and nmoles/minutes calculated.

3.14 Analysis of hormones

Insulin, C-peptide, active ghrelin, glucagon, active GLP-1, total GIP and leptin were measured in plasma using a multiplex system (Milliplex MAP Kit, Human Metabolic Hormone, Milliplex MAP Kit Human Serum Adipokine, Merck Millipore, Schwalbach, Germany, Bio-Plex Pro diabetes assays, Bio-Rad, München, Germany) based on the Luminex®xMAP® technology. For the healthy group, the multiplex system was applied from Millipore. Hormones from the prediabetic group were analysed by the multiplex system from Biorad because of different ranges. These immunoassays enable simultaneous measurements of multiple analytes in the same sample by using color-coded beads coated with a specific biotinylated capture antibody. After conjugation of Streptavidin, polyethylene microspheres pass through a laser which excites the internal dyes marking the microsphere set. A second laser excites the fluorescent dye on the reporter molecule. Finally digital-signal processors identify each individual microsphere and quantify the result of its bioassay based on fluorescent reporter signals.

3.15 Quantification of amino acids and derivatives

For analysis of amino acids and their derivatives in plasma of the healthy group, the iTRAQ® method (AA45/32™Phys REAG Kit, Applied Biosystems, Foster City, USA) was applied. Sample preparation was performed according to the manufacturer's protocol. 10µl of 10% sulfosalicylic acid was added to 40µl of plasma to precipitate the proteins. The sulfosalicylic acid contains 400 pmol/µl norleucine as an internal control for the sample preparation. The samples were vortexed and centrifuged for 2.5 min at 10000 x g (Galaxy 14 D, VWR, Darmstadt, Germany). 10µl of the supernatant was diluted with 40µl labeling buffer (borate buffer, pH 8.5, containing 20 pmol/µl norvaline) and centrifuged. Afterwards, 10µl of the dilution was transferred to a new tube and 5µl iTRAQ® 115 reagent was added. Samples were vortexed, centrifuged and incubated for 30 min at room temperature. 5µl hydroxylamine (1.2%) was added to stop the reaction, and the samples were dried in a centrifugal vacuum concentrator (SPD 111V SpeedVac, Thermo Savant, Germany) for 1h. Samples were redissolved by adding of 32µl iTRAQ reagent 114-labled standard mix (containing 5 pmol of each amino acid per µl except for L-cystine (2.5 pmol/µl)) and diluted with 128µl mobile Phase A (water with 0.1% formic acid and 0.01% heptafluorobutyric acid). Afterwards, 10µl of the prepared sample were automatically injected (autosampler HTC PAL, CTC Analytics AG, Zwingen, Switzerland) and the derivatised amino acids were separated at 50°C on an AAA C18 column, 4.6 x 150 mm (Applied Biosystems, Foster City, USA) with the LC system (Agilent Technologies 1200 Series, Agilent Technologies, Germany). The mobile phase consisted of 0.1% formic acid and 0.01% heptafluoro-butyric acid dissolved in deionized water (mobile phase A) or acetonitrile (mobile phase B containing 0.1% formic acid and 0.01% heptafluorobutyric acid) respectively. Flow rate was 800µl per min. Mass analysis was performed using the 3200QTRAP LC/MS/MS system (Applied Biosystems, Foster City, USA) operating in a multiple reaction-monitoring (MRM) mode. For quantification, the Analyst® 1.5 Software (Applied Biosystems, Foster City, USA) was used. Norleucine and norvaline served as quality control, for workflow efficiency and for labeling efficiency.

For the analysis of amino acids and their derivatives in the samples of the prediabetic group the aTRAQ method was applied (aTRAQ™ Reagent Kit 200 Assay, ABSciex, Foster City, USA). Sample preparation and analysis is essentially the same as for the iTRAQ described above with only minor differences: Compared to the iTRAQ-labeling method, amino acids and derivatives were derivatised with an aTRAQ-reagent (mass121). The internal standard mix was aTRAQ-labeled with a mass of 113. In contrast to the iTRAQ method, the mobile phase B was methanol containing 0.1% formic acid and 0.01% heptafluorobutyric acid.

3.16 Analysis of acetaminophen and acylcarnitines

A whatman filterpaper 903 was punched out (ø 6 mm) and placed in a 0.45 mm Millipore 96 well PTFE filter plate (multiscreen Solvinert) which was fixed on a 96 deep well plate (Nunc). Plasma was diluted 1 + 19 with LCMS-water containing 0.045% NaCl. 10µl of the diluted plasma was pipetted on the 6 mm diameter whatman 903 filterpaper spot. The plasma was dried under vacuum at room temperature for 45 min. The methanol standard mixture contained 40µL/mL of a neonatal Standard (Chromsystems, München, Germany) and 50 ng/mL of d4-acetaminophen from LGC Standards (LGC Standards GmbH, Wesel, Germany). 250µL of the methanol standard mixture were added on each spot and sealed with a silicon mat. The plate was agitated at 350 rpm for 30 minutes at 25°C and centrifugated at 1400g. Afterwards, 250µl of the methanol standard mixture was pipetted again on the spots and agitated for 5 minutes at 25°C and centrifugated. Afterwards, the filter plate was removed and the deep 96 well plate was sealed. 20µl of the samples was injected with a CTC autosampler in FIA mode into an AB Sciex 5500 QTRAP with Analyst data acquisition software. The running solvent was LCMS methanol containing 5 mM ammonium acetate. The running settings were 25µl/min and source temperature was 200°C. Settings for the analysis of paracetamol were adapted from the publication of Li (Li et al. 2010). The FIA peak was 1 min and each analyte peak contained 15 datapoints. The AB Sciex Multiquant Software was applied for the analysis of FIA peaks.

3.17 Calculations and statistical analyses

Data were all expressed in means of fold changes (fc) and SEM. AUC of fold change data were calculated from baseline using Graph Pad PRIMS Version4. To compare the AUC of analytes, a repeated One-way ANOVA was performed. For comparison of the test drinks and effects over the 240 min time period, a two-way ANOVA with repeated measures using the MIXED model setting with "meal" as a fixed effect, "analyte" as a covariate and "subject" as a random effect was applied under SAS version 9.2 (SAS Institute Inc, Cary, NC). All *P* values were adjusted according to Tukey-Kramer's multiple group comparison procedure. *P* values < 0.05 were considered to be statistically significant. Pearson's correlation test was performed using Graph Pad PRISM Version4; (GraphPad Software, Inc. La Jolla, CA, USA). For the correlation of the amino acids derived from the test drinks and maximum plasma amino acids, mean plasma values were calculated for each time point within healthy and prediabetic subjects, respectively, and their correlations were analyzed using Kendall's tau. Correlations were performed using the software R (version 3.0.2) and the R package corrplot. For the analysis of the maximum concentration and time to maximum of hydroxyproline and acetaminophen, the Bateman function was applied with R. We used the nl2sol algorithm with the function nls from the package stats. For calculation of the inflexion points of the hydrogen exhalation curves, data were calculated using the Boltzmann formula in SAS version 9.2 and Wolfram Mathematica 8, (Wolfram Centre, Oxfordshire UK). To compare the inflexion points of the test drinks, a repeated One-way ANOVA in SAS was performed.

4 Results

4.1 Alterations in glucose levels

As expected, blood glucose concentrations increased after ingestion of the test drinks and decreased below baseline levels after 60 to 90 min in healthy volunteers. Fold changes of venous blood glucose concentrations and interstitial glucose evoked by the different test drinks in both groups are shown in **Figure 8 A**, **C** and **B**, **D**, respectively. Absolute values from both groups are displayed in **Table 8**. Baseline levels of blood glucose were not different between volunteers. The graphs displaying absolute blood glucose concentration profiles from both groups are shown in the appendix (**Figure 36 A, C** and **B, D,** respectively). At 30 min, the increases of blood glucose were significantly lower after the WPI, CP and GMP drinks compared to the MD19 in healthy volunteers ($P < 0.0001$, for all). Peaks of venous blood glucose from the healthy group are summarized in the **appendix Table 43**. All test drinks suppressed AUC of venous blood glucose significantly compared to the control drink within 120 min ($P < 0.0001$, **Figure 9 A**). Additionally, area under the curve values over 240 min of the fold-changes of blood glucose were reduced after the GMP and the CP drinks ($P = 0.0073$, $P = 0.0413$, respectively, **Figure 9 B**) but not after the WPI drink when compared to MD19.
In the prediabetic group, the peaks of venous blood were significantly lower after all test drinks compared to the MD19 test ($P < 0.001$, for all) (**Figure 8 B**) with the GMP drink eliciting strongest decrease of blood glucose levels at 180 min. Interstitial glucose peaks were shifted by 5 min after the WPI and CP drinks compared to venous blood glucose peaks (**Figure 8 D**). The AUC of venous blood glucose was reduced by 18% after the GMP drink, by 15% after the CP drink and by 11% after the WPI drink compared to MD19 over the 240 min time course ($P < 0.001$, respectively). Only in the prediabetic group, the GMP drink elicited a significant difference in the AUC of venous blood glucose compared to the WPI drink (8%, $P = 0.048$, **Figure 9 B**).

No significant difference between drinks was observed in the interstitial glucose concentrations. This may be due a rather low number of subjects equipped with the Freestyle Navigator (**Figure 8 C**) in the group of healthy volunteers. A small shift in the maximal glucose peak of the interstitial glucose was observed when compared to the venous blood glucose, which for all test drinks occurred at 40 min.

In the prediabetic group, the interstitial glucose peak reached the maximum 10 min later in case of MD19 and 30 min later in case of the GMP drink when compared to venous levels. Significant differences of interstitial glucose peak values were observed after all test drinks when compared to MD19 ($P < 0.0001$, for each). The AUC after 120 and 240 min of the interstitial glucose concentration curves were significantly different for the WPI, CP and GMP drinks compared to the MD19 control (120 min: $P < 0.0001$ for each, 240 min: $P = 0.0039$, $P = 0.0002$, $P < 0.0001$, respectively). Correlations between venous blood glucose and interstitial glucose concentrations are shown in **appendix Figure 47-49**.

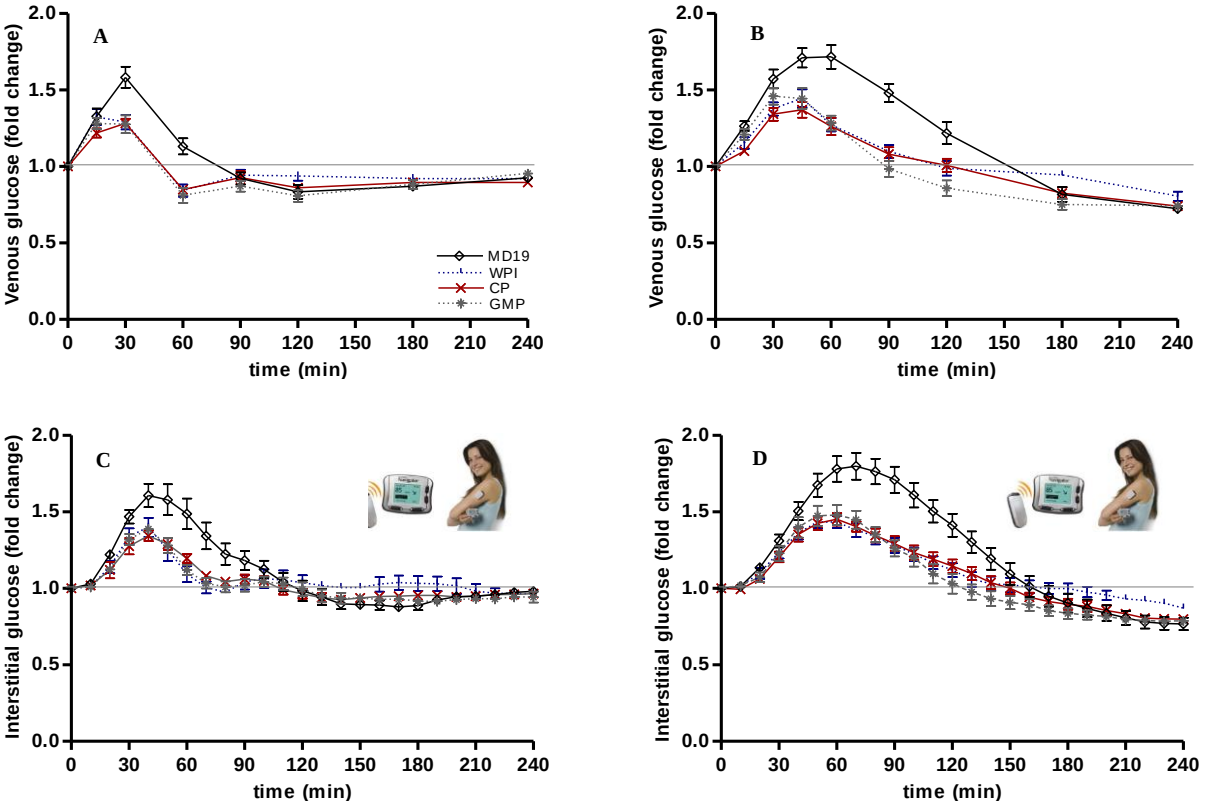


Figure 8: Means of fold changes of venous blood and interstitial glucose concentrations over time ± SEM**.** The effects after 50g maltodextrin19 or either 50g protein or peptide ingestion (whey protein isolate, Na-caseinate, GMP) together with 50g MD19 in healthy **(A:** n = 15, **C:** n = 7**)** and prediabetic subjects (**B:** n = 15, **D:** n = 14) on venous blood glucose (**A, B**) and interstitial glucose (**C, D**) Data are expressed in means of fold changes ± SEM. **Healthy group** (**A**): Venous blood glucose: CP drink and GMP drink compared to MD19 drink (P = 0.024, P = 0.0013, respectively), (**C**): Interstitial glucose: not significant, **Prediabetic group** (**B**): Venous blood glucose: WPI drink, CP drink and GMP drink compared to MD19 drink (P < 0.0001, for all), (**D**): Interstitial glucose: WPI drink, CP drink, GMP drink compared to the MD19 drink (P = 0.0264, P = 0.0004, P < 0.0001, respectively). Significance was analyzed by two way repeated-measures ANOVA and Tukey Kramer post hoc test.

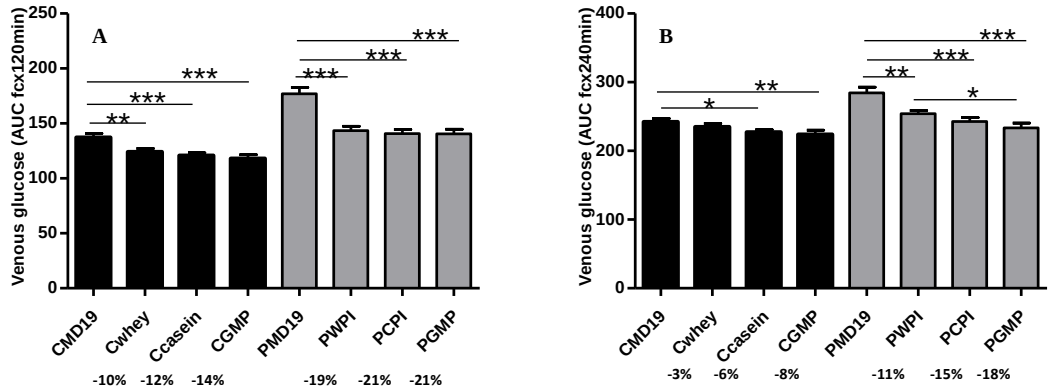


Figure 9: Area under the curve of means of venous blood glucose ± SEM after 120 min **(A)** and after 240 min **(B)** which were calculated from fold change data. The relative changes shown below the baseline are related to the MD19 test drink. C = Control group, *P* = Prediabetic group: N = 15 for each group. Significance of the AUC was analyzed by One-Way ANOVA with repeated measures followed by Tukey Kramer post hoc test. Significance: *** P < 0.001, ** P < 0.01, * P < 0.05

Table 8: Absolute venous blood glucose concentrations

Healthy group: Venous blood glucose in mg/dl				
min	**WPI**	**CP**	**GMP**	**MD19**
0	75.49±1.75[a]	80.65±1.90[a]	80.75±2.11[a]	77.54±1.78[a]
15	99.73±4.37[a]	98.01±2.71[a]	102.99±2.72[a]	103.09±4.65[a]
30	97.26±3.88[a]	103.29±3.09[a]	102.62±4.86[a]	122.21±5.26[b]
60	63.61±3.41[a]	68.37±2.02[a]	64.83±3.35[a]	87.17±3.53[b]
90	71.50±3.46[a]	74.78±3.88[a]	70.16±3.03[a]	71.04±2.59[a]
120	70.87±2.84[a]	69.51±2.53[a]	64.79±2.59[a]	64.39±3.26[a]
180	69.58±2.16[a]	72.21±1.90[a]	70.86±2.27[a]	67.18±1.62[a]
240	69.15±1.71[a]	71.89±1.66[a]	76.59±2.19[a]	71.43±1.19[a]

Prediabetic group: Venous blood glucose in mg/dl				
min	**WPI**	**CP**	**GMP**	**MD19**
0	102±2.84[a]	102±3.23[a]	98.47±3.07[a]	100.13±2.94[a]
15	116±11.36[a]	112±3.43[a]	118.27±3.59[a]	126.67±4.92[a]
30	139±17.04[ab]	136±3.25[a]	142.13±4.41[ab]	156.87±6.58[b]
45	146±21.65[a]	138±3.25[a]	141.07±6.25[a]	170.87±7.01[b]
60	129±18.61[a]	128±5.21[a]	125.87±5.02[a]	171.93±8.89[b]
90	112±20.23[a]	110±4.40[a]	96.53±5.54[a]	148.27±7.36[b]
120	101±21.44[a]	103±4.95[a]	83.20±3.46[b]	121.13±6.87[c]
180	96±12.79[a]	84±3.82[ab]	73.53±3.45[b]	80.93±4.55[b]
240	82±11.81[a]	75±2.64[a]	72.47±2.44[a]	71.93±2.03[a]

Means with different letters are significantly different, $P < 0.05$.

Table 9 shows the difference of venous blood glucose of the healthy versus the prediabetic group in responses to the test drinks. Most interestingly, the difference in the AUC of venous blood glucose after intake of the protein containing drinks over the 240 min time course is much less pronounced than the difference in response to the MD19 drink between the prediabetic group and the healthy group.

Table 9: Difference of venous blood glucose of the prediabetic group compared to the healthy group

	AUC 90 min	*P* value	AUC 240 min	*P* value
WPI	+17%	0.0001	+8%	0.0056
CP	+16%	0.0004	+7%	0.0157
GMP	+22%	0.0002	+4%	0.3113
MD19	+23%	0.0001	**+17%**	**0.0008**

4.2 Changes in systemic hormone levels

4.2.1 Insulin, C-peptide and glucagon changes after intake of different test drinks

Plasma insulin concentration was similarly increased after the WPI and CP drinks with a maximum level reached at 30 min (**Figure 10 A**) in the healthy group. Remarkably, the plasma insulin peak was lower after GMP compared to the MD19 drink and was also significantly lower compared to that after the WPI and the CP drinks ($P = 0.0073$, $P = 0.0370$, respectively).

The 240 min AUC of plasma insulin was strongly elevated after the WPI drink compared to the MD19 drink (+57%, $P = 0.0034$) and those of WPI and additionally CP were significantly increased compared to GMP in the healthy group (+100%, +69%, $P < 0.0001$, $P = 0.0061$, respectively, **Figure 13 A**).

In prediabetics, plasma insulin concentrations peaked at 60 min after the WPI and GMP and at 90 min after the CP drink (**Figure 10 B**). The peak of insulin after the WPI drink and the CP drink were significantly higher to that after the MD19 drink ($P = 0.0073$, $P = 0.0196$, respectively).

The AUC of plasma insulin was also significantly higher for the WPI drink and the CP drink when compared to MD19 drink (+158%, +117%; *P* = 0.0011, *P* = 0.0146 respectively, **Figure 13 A**) in the prediabetic group. The WPI drink induced also a significantly higher AUC of insulin than did the GMP drink (*P* = 0.0170).

Plasma C-peptide concentrations increased strongest after WPI followed by GMP and CP drinks with maximum concentrations reached at 30 min (**Figure 10 C)** in the healthy group without significant differences concerning peaks. However, AUC values of C-peptide were increased by 31% after WPI compared to MD19 (*P* = 0.0391) and also significantly different to those after GMP (*P* = 0.0209, **Figure 13 B**). Correlations are demonstrated in the **appendix Table 61** showing strong associations between C-peptide and levels of arginine, asparagine, glycine, leucine, methionine, ornithine and serine blood levels for all protein containing drinks in healthy volunteers.

In the prediabetic group, plasma C-peptide peaked at 60 min after GMP and at 90 min after WPI and CP (**Figure 10 D**) but peaks were not significantly different. However, the AUC of C-peptide was elevated after the WPI and CP drinks compared to MD19 (+23%, +19%, *P* = 0.0032, *P* = 0.0215 respectively, **Figure 13 B**). It was also different after the WPI drink compared to the GMP drink (*P* < 0.05).

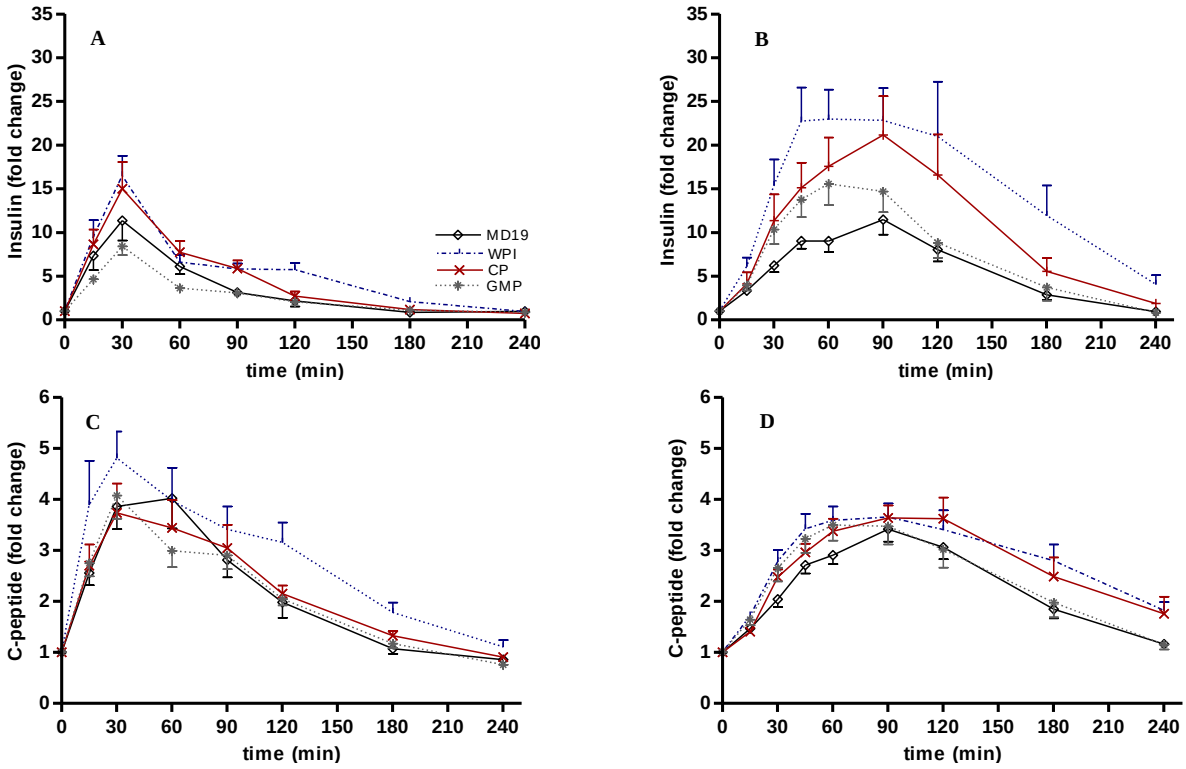


Figure 10: Changes in plasma levels of insulin and plasma C-peptide in the healthy group (A: n = 15, C: n = 15) **and the prediabetic group** (B: n = 11-15, D: n = 15). Insulin and C-peptide were measurd in a Multiplex System. Significant differences were observed for insulin in the healthy group (**A**): WPI drink and CP drink compared to GMP drink: (*P* = 0.0003, *P* = 0.0167, respectively); WPI drink compared to MD19 drink (P = 0.0078), in the prediabetic group (**B**): WPI drink, CP drink, GMP drink compared to the MD19 drink (P = 0.0005, P = 0.0017, P = 0.04, respectively), WPI drink compared to the GMP drink (P = 0.0175). (**C**) C-peptide in the healthy group did not reach statistical power. C-peptide in the prediabetic group (**D**): WPI drink compared to the MD19 drink (P = 0.0048). Significance was analyzed by two way repeated-measures ANOVA with treatment effects and treatment x time interactions and Tukey Kramer post hoc test.

The hepatic insulin extraction index as the ratio between C-peptide and insulin was significantly lower after WPI and CP compared to GMP (-32%, $P = 0.0213$, -34%, $P = 0.0267$, respectively, **Table 10**) in the healthy group. The insulin index as a surrogate for the effectiveness of insulin to act on glucose disposal indicate that much more insulin was present in the circulation to reduce glucose concentrations after WPI and CP compared to GMP (+92%, $P = 0.035$, +67%, $P = 0.0383$, respectively, **Table 11**). In the prediabetic group, the hepatic insulin extraction index was significantly lower after WPI and CP compared to MD19 (-52%, $P = 0.0005$, -35%, $P = 0.0255$) which was confirmed by the insulin index (+200%, $P = 0.0027$, +149%, $P = 0.0241$ **Table 10, Table 11**).

Table 10: Hepatic insulin extraction index of the healthy and prediabetic group

	WPI	CP	GMP	MD19
Healthy group				
AUC C-peptide	678.16±74.92^{a}	530.37±51.00^{ab}	502.50±31.62^{b}	517.10±51.00^{b}
AUC Insulin	1304.57±115.53^{a}	1099.71±152.31^{ab}	648.76±57.20^{c}	918.71±187.64^{b}
AUC C-peptide/Insulin	0.58±0.08^{a}	0.56±0.06^{a}	0.85±0.08^{b}	0.75±0.12^{a}
Prediabetic group	**WPI**	**CP**	**GMP**	**MD19**
AUC C-peptide	688.58±54.30^{a}	660.66±57.42^{a}	592.17±56.28^{b}	556.89±31.01^{b}
AUC Insulin	3914.33±737.56^{a}	3100.08±606.64^{ab}	1899.25±234.59^{ab}	1459.28±181.25^{b}
AUC C-peptide/Insulin	0.23±0.02^{a}	0.31±0.05^{ab}	0.38±0.04^{bc}	0.48±0.05^{c}

^{a,b,c} Mean values with different letters are significantly different between treatments ($P < 0.05$).
Significance was analyzed by One-Way ANOVA with repeated measures followed by Tukey Kramer post hoc test.
Healthy group, n = 15, prediabetic group n = 11.

Table 11: Insulin index of the healthy and prediabetic group

	WPI	CP	GMP	MD19
AUC Insulin	1304.57±115.53^{a}	1099.71±152.31^{ab}	648.76±57.20^{c}	918.71±187.64^{bc}
AUC Glucose	235.06±18.88^{a}	227.31±13.99^{a}	223.73±24.48^{a}	242.07±19.16^{b}
AUC Insulin/Glucose	5.55±0.52^{a}	4.83±0.73^{a}	2.90±0.23^{b}	3.80±0.70^{a}
Prediabetic group	**WPI**	**CP**	**GMP**	**MD19**
AUC Insulin	3914.33±737.56^{a}	3100.08±606.64^{ab}	1899.25±234.59^{ab}	1459.28±181.25^{b}
AUC Glucose	253.98±4.71^{a}	242.69±5.86^{a}	233.54±6.73^{a}	284.28±8.38^{b}
AUC Insulin/Glucose	15.41±2.86^{ac}	12.77±2.50^{bc}	8.13±1.10^{b}	5.13±0.61^{b}

^{a,b,c} Mean values with different letters are significantly different between treatments ($P < 0.05$).
Significance was analyzed by One-Way ANOVA with repeated measures followed by Tukey Kramer post hoc test.
Healthy group, n = 15, prediabetic group n = 11.

Plasma glucagon concentrations are shown in **Figure 11**. Strongly elevated levels were observed for all 3 test drinks when compared to MD19 ($P < 0.05$) in which glucagon levels fell below baseline but no differences were found between the 3 test proteins. All peak hormone levels are summarized in the **appendix Table 43** and correlations of glucagon with amino acids levels are compiled in the **appendix Table 63.** The AUC of glucagon levels were highest after GMP (+115%) followed by WPI (+92%) and CP (+91%) and were significantly different to MD19 ($P = 0.0028$, $P = 0.0226$, $P = 0.0216$, respectively, **Figure 11C**).

In the prediabetic group, glucagon peaked at 45 min after the WPI drink, whereas peaks in case of the CPI and GMP drink were obtained at 30 min (**appendix Table 44).** Correlations between glucagon and amino acids are presented in the **appendix Table 64**. Strong correlations were especially observed between glucagon and levels of isoleucine, leucine, methionine, ornithine and phenylalanine after the

WPI and the CP drinks. The AUC of glucagon was highest after the WPI drink (+90%) followed by the CP drink (+75%) and the GMP drink (+66%) and significantly different to the MD19 drink (P = 0.0003, P = 0.0026, P = 0.0082, respectively, **Figure 11 C**). The insulin/glucagon ratio is presented in **Figure 11 D.**

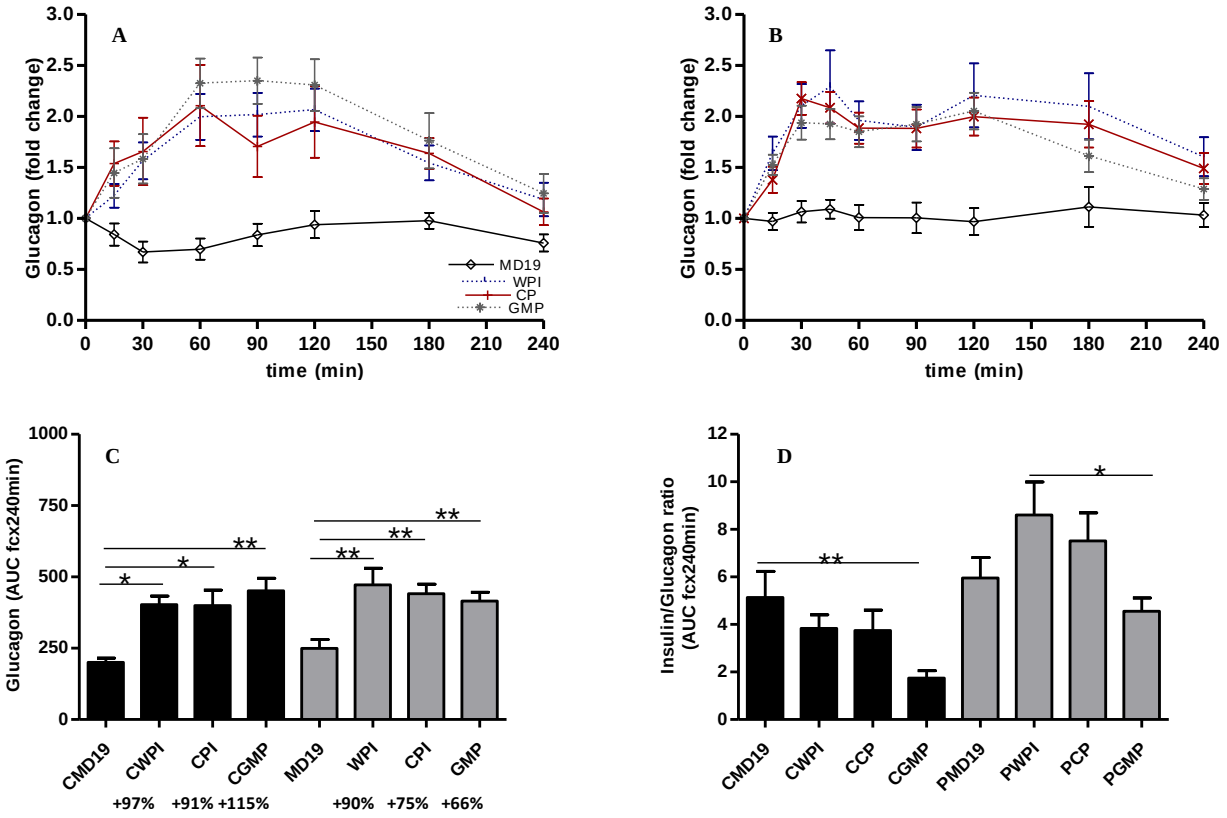


Figure 11: Plasma glucagon and insulin/glucagon ratio over 240 min expressed in means of fold changes ± SEM in the healthy group (**A**, **C**: n = 10) and the prediabetic group (**B**, **C**: n = 13-14). Significant differences between diets were observed in the **healthy group**: WPI drink, CP drink, GMP drink compared to the MD19 drink (P = 0.0003, P = 0.0026, P < 0.0001, respectively) and in the **prediabetic group**: WPI drink, CP drink, GMP drink compared to MD19 (P = 0.0005, P = 0.0025, P < 0.0001, respectively). For the AUC (**C**, **D**): C = Healthy group n = 10, P = Prediabetic group: n = 12-15. Significance of the AUC was analyzed by One-Way ANOVA with repeated measures followed by Tukey Kramer post hoc test. The percental comparison below the baseline is related to the MD19 test drink. Significance: *** P < 0.001, ** P < 0.01, * P < 0.05

4.2.2 Alterations of incretin hormones and DPPIV after intake of different test drinks

Total plasma GIP revealed a first peak at 30 min for all test drinks (**Figure 12 A)** in healthy volunteers. After the WPI and the GMP drink a similar biphasic pattern was observed with a second peak at around 90 min. The first peak of GIP after the WPI drink was significantly higher than to that after the GMP (P < 0.05) and the CP drink (P < 0.05). AUC values of GIP were significantly higher after the WPI than after MD19 (92%, P = 0.0002), CP (45%, P = 0.0221) and GMP (70%, P = 0.0014, **Figure 13 C**).

In the prediabetic group, total plasma GIP peaked at 30 min after GMP and CP and at 45 min after the WPI and MD19 drinks without significant differences (**Figure 12 B**). Though, the AUC of total GIP

was elevated by 53% and 51% after the WPI and CP drinks, respectively as compared to MD19 drink ($P < 0.05$, for both, **Figure 13 C**).

Strong correlations were found between GIP and C-peptide levels after the WPI, CPI and GMP drink (r = 0.96, $P = 0.0002$, $R^2 = 0.91$; r = 0.9782, $P < 0.0001$, $R^2 = 0.9569$; r = 0.9311, $P = 0.0008$, $R^2 = 0.8670$, respectivley) in healthy volunteers. Furthermore, GIP levels correlated very well with selected plasma amino acid levels (**appendix Table 65** and **appendix Figure 44 - 46**). Also in the prediabetic group, a strong correlation was found between GIP and C-peptide after the WPI drink, the CP drink and the GMP drink (r = 0.8909, $P = 0.0013$, $R^2 = 0.7937$; r = 0.8673, $P = 0.0025$, $R^2 = 0.7523$; r = 0.8492, $P = 0.0038$, $R^2 = 0.7212$). Moreover, some hormones correlated with GIP (**appendix Table 52)** and also selected amino acid levels correlated well with some of the plasma hormones (**appendix Table 66, appendix Figure 47, 48).**

Active plasma GLP-1 peaked at 15 min after intake of all test drinks **(Figure 12 C)** in healthy volunteers. The highest peak was found after WPI intake followed by CP and GMP over MD19. Active plasma GLP-1 was strongly elevated after the WPI drink over the 240 min time period. However, no significant differences of GLP-1 levels or AUC values could be observed between the 3 test and control drinks (**Figure 13 D)**.

In the prediabetic group, plasma GLP-1 (analyzed with the Multiplex system) peaked at 30 min after CP, GMP and MD19 and at 45 min after WPI (**Figure 12 D**). One subject of the prediabetic group was excluded from GLP-1 analysis because values deviated more than 2 fold of the standard deviation of the mean. When determined with the high sensitivity ELISA, GLP-1 peaked at 30 min after the protein containing drinks without any significant differences and at 45 min in case of MD19 (**Figure 12 E**). In the AUC, all 3 protein containing drinks caused significantly higher levels than the MD19 drink ($P < 0.0001$, $P = 0.0015$, $P < 0.0001$, **Figure 13 E**). Significant differences in the AUC of GLP-1 could also be observed between the WPI and the CP drinks ($P = 0.0404$). Interestingly, DPPIV activity in plasma rapidly decreased after the CP drink at 120 min (**Figure 12 F**). However, no statistical difference in DPIV activity was observed between the test drinks.

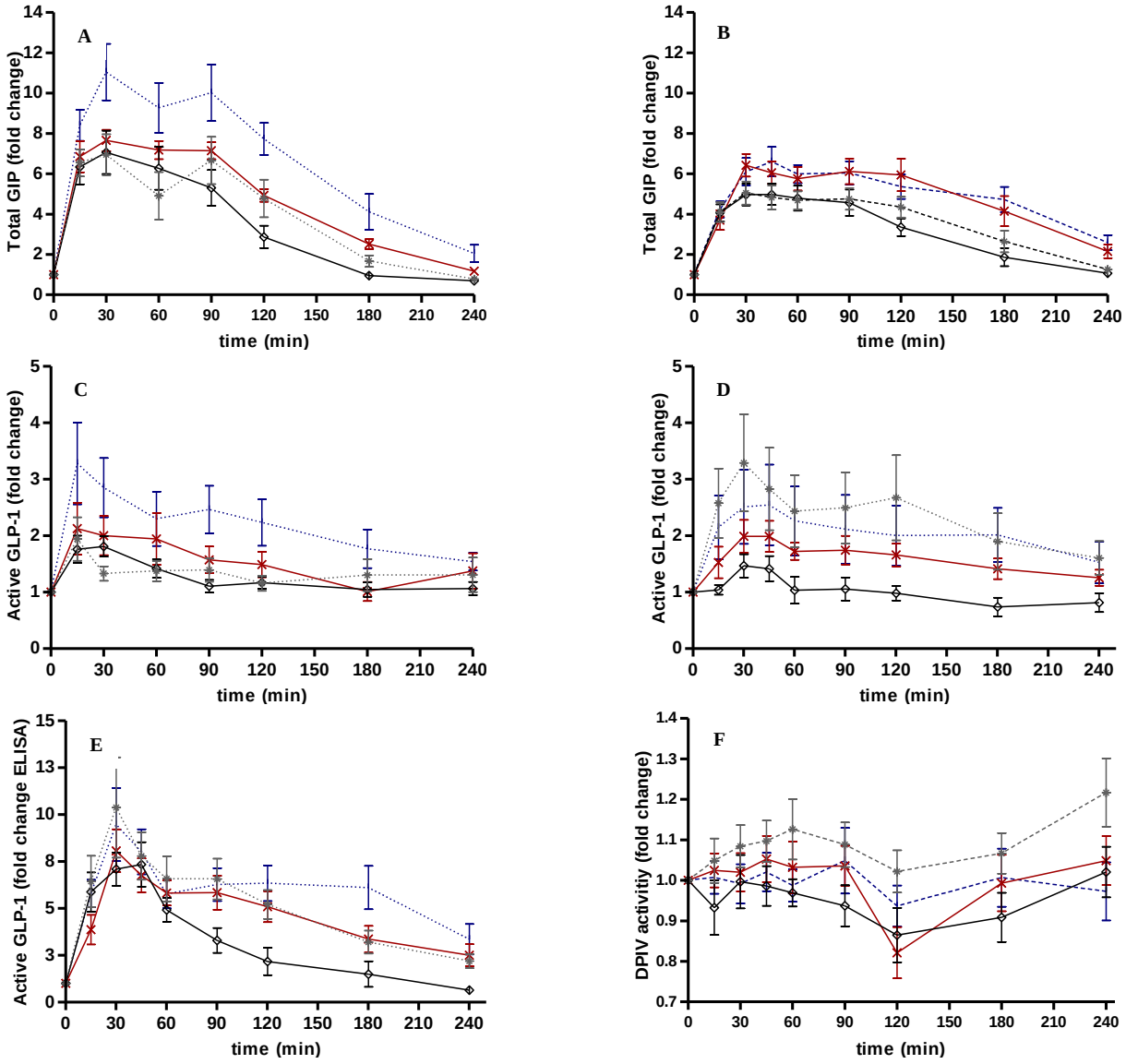


Figure 12: Total plasma GIP and GLP-1 in the healthy group (**A:** n = 15, **C:** n = 10-14) **and additionally DPPIV in the prediabetic group** (**B**: n = 14-15, **D**: n = 6-11, **E:** n =15, **F:** n = 15). (**A**): WPI drink compared to the CP drink, GMP drink and the MD19 drink ($P = 0.0308$, $P = 0.0127$, $P < 0.0001$, respectively), total plasma GIP in the prediabetic group (**B**): WPI drink and CP drink compared to the MD19 drink ($P = 0.0199$, $P = 0.0332$, respectively). (**C**): No significant difference was observed for active plasma GLP-1 in the healthy group. (**D**): In the prediabetic group, no statistical power could be observed for GLP-1 with the Multiplex System. (**E**): However, GLP-1 was significantly different after the WPI drink, CP drink, GMP drink compared to the MD19 drink with the high sensitivity ELISA ($P < 0.0001$, $P = 0.0015$, $P < 0.0001$, respectively). (**F**): Plasma DPPIV activity in prediabetic volunteers was statistically not different between treatments. Data are expressed in means ± SEM. Significance was analyzed by two way repeated-measures ANOVA with treatment effects and treatment x time interactions and Tukey Kramer post hoc test.

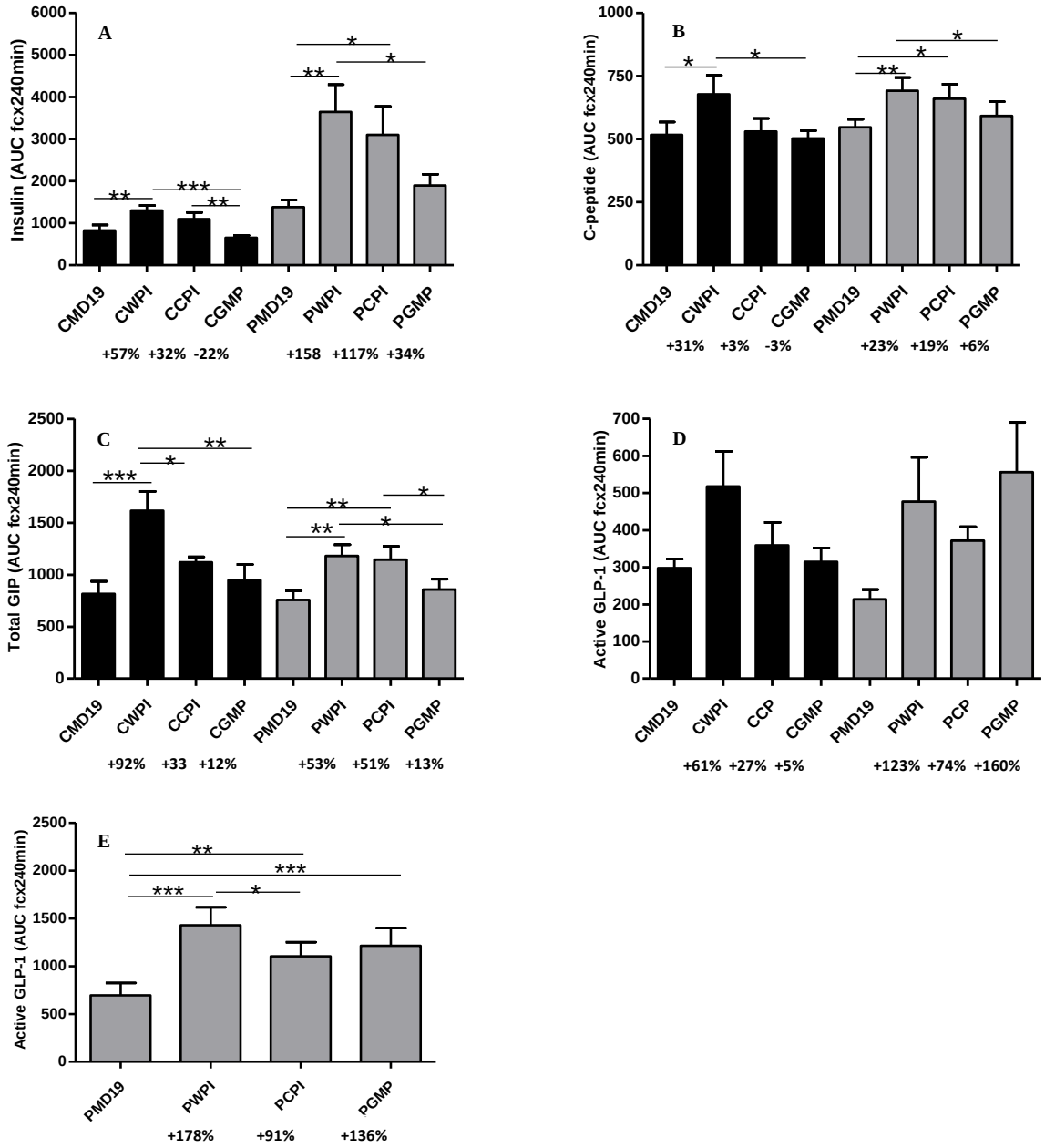


Figure 13: Area under the curve of plasma insulin, C-peptide, total GIP and active GLP-1 in both groups. Data are expressed in means ± SEM **A:** Plasma insulin (C: n = 15, P = 12-15), **B:** Plasma C-peptide (C: n = 15, P = 15), **C:** total plasma GIP (C = 15, P = 14-15), **D:** Active plasma GLP-1 (C: n = 10-14, P = 6-11) **E:** Active plasma GLP-1 ELISA: (n = 15). The percental comparison below the baseline is related to the MD19 test drink. C = Control group, P = Prediabetic group. Significance of the AUC was analyzed by One-Way ANOVA with repeated measurements followed by Tukey Kramer post hoc test. Significance: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$

4.3 Influence of the test drinks on gastric emptying and oro cecal transit time

Hydroxyproline was chosen as an extrinsic marker for the velocity of amino acid absorption since its blood levels are not to changed in plasma after an OGTT (own studies). The gold standard for the assessment of gastric emptying is scintigraphy (Smout et al. 1994) which requires invasive techniques. Therefore, administration of acetaminophen with subsequent plasma analysis is frequently used to

assess kinetics of gastric emptying and thus we used acetaminophen in healthy volunteers for GMP and for all test drinks in the prediabetic group.

In healthy volunteers, the time to reach maximum hydroxyproline concentrations in plasma was slightly different between the WPI drink (119 ± 12 min, 87 µMol) and the MD19 drink (97 ± 6 min, 86 µMol, $P = 0.0113$) but not between the WPI and the CP drinks (102 ± 10 min, 90 µMol). Maximal plasma concentrations were also not different (**Figure 14 A**).

In prediabetics, time to maximum acetaminophen concentrations was not different between the WPI (132 ± 21 min, 21 ± 1.18 µg/ml), the CP (127 ± 12 min, 22 ± 1.23 µg/ml) and the GMP drink (120 ± 14 min, 26 ± 1.41 µg/ml, respectively). However, time to reach maximum concentrations was significantly different between 3 test drinks when compared to MD19 (82 ± 9 min, 30 ± 1.85 µg/ml, $P = 0.0011$, $P = 0.0034$, $P = 0.0175$ respectively) (**Figure 14 C**). For gastric emptying, a strong correlation was found between the calculated AUC of plasma acetaminophen and the percentage of food emptied from stomach as assessed by scintigraphy (r = 0.94, Nimmo et al. 1975). Therefore, we calculated the AUC of acetaminophen and observed the fastest gastric emptying after the MD19 test drink followed by the GMP, the CP and the WPI drinks in the prediabetic group (**Figure 14 D**).

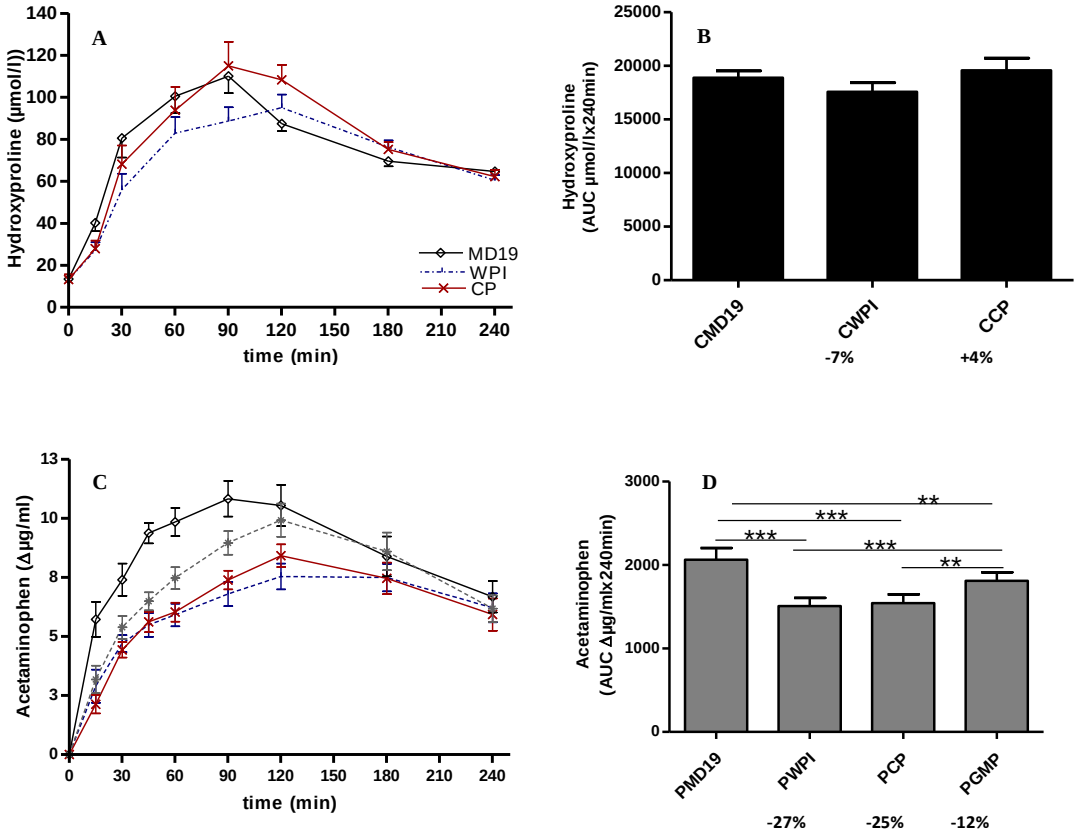


Figure 14: Plasma hydroxyproline and plasma acetaminophen changes over the 240 min time course. Hydroxyproline showed no statistical difference after intake of the WPI drink, CP drink and the MD19 drink when presented as means ± SEM or AUC (**A**, **B**, n = 12). Significant difference in gastric emptying was observed in the prediabetic group after the WPI drink, the CP drink containing acetaminophen as a marker compared to the MD19 drink and the GMP drink over the 240 min time course (**C**, n = 15) and calculated as AUC ($P < 0.01$, for each, **D**, n = 15). Gastric emptying differd also significantly after the GMP drink compared to the MD19 drink ($P = 0.0007$). No significant difference could be observed in gastric emptying after intake of the WPI drink and the CP drink.

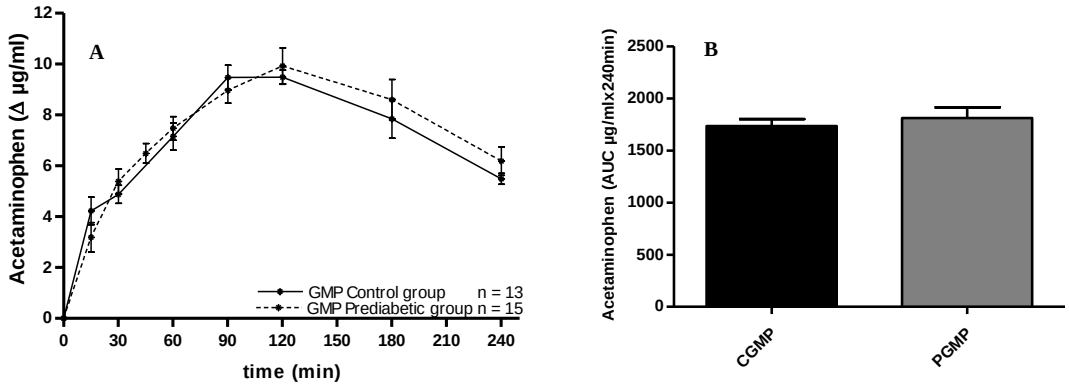


Figure 15: Gastric emptying between the healthy and prediabetic group after the GMP challenge (**A, B**). Significance of the AUC was analyzed by One-Way ANOVA with repeated measures followed by Tukey Kramer post hoc test. Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

The AUC of acetaminophen was discriminative after the GMP drink compared to the MD19 drink, the CP drink and the WPI drink ($P < 0.0029$, $P < 0.0015$, $P < 0.0003$, respectively) whereby the AUC of acetaminophen after the WPI drink and the CP drink were also different to the MD19 drink ($P < 0.0001$, $P < 0.0001$, respectively; **Figure 14 D**). No difference could be observed in gastric emptying between the healthy group and the prediabetic group when receiving GMP (**Figure 15 A, B**).

To simulate gastric handling of the different test drinks in stomach, we determined *in vitro* the precipitation by incubation of the different test drinks with 0.2% HCl and 0.5% pepsin (**Figure 16 A**) and by weight determination of dried precipitates (**Figure 16 B**). The buffering capacitiy of the CP drink was found to be higher in the pH range between 4.0 and 6.0 compared to the WPI drink but was not different between 4.0 and 2.0. The GMP drink buffered more effectively in the range between 2.5 and 5.0 compared to the WPI and the CP drinks (**Figure 16 C**).

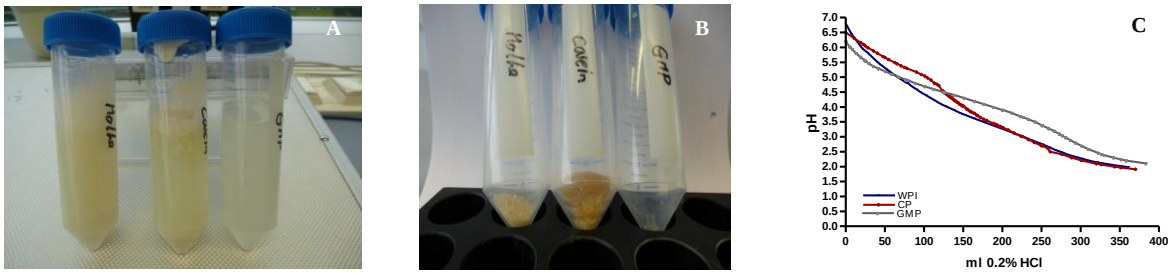


Figure 16: Precipitation of the milk fractions in the presence of MD19 after incubation with 0.2% HCl and 0.5% pepsin at 37°C for 1 h (**A**). Precipitates were dried and weighted (**B**). Determiniation of buffer capacity by titration of the milk fraction drinks with 0.2% HCl without pepsin (**C**).

Apparent, oro-cecal transit times of the test drinks were determined by calculation of the inflexion points of the hydrogen exhalation curves. Here, the GMP drink showed the fastest transit time with 140 min ± 23 min, followed by the MD19 drink with 142 ± 17 min. The WPI drink showed the slowest transit with 179 ± 27 min followed by the CP drink with 151 ± 24 min. Transit time of the

WPI drink was significantly different compared to the CP, GMP and MD19 ($P = 0.0014$, $P < 0.0001$, $P < 0.0001$, respectively) in healthy volunteers (**Figure 17 A, Table 12**).

Noticeable, the hydrogen exhalation was strongly increased after the GMP drink in the prediabetic group. Calculation of the inflexion point demonstrated here that the transit time was fastest after the MD19 test drink (158 ± 21 min) but was similar after the protein containing drinks (174 ± 36 min, 174 ± 40 min, 175 ± 25 min, respectively; **Figure 17 B, Table 12**) without significant differences.

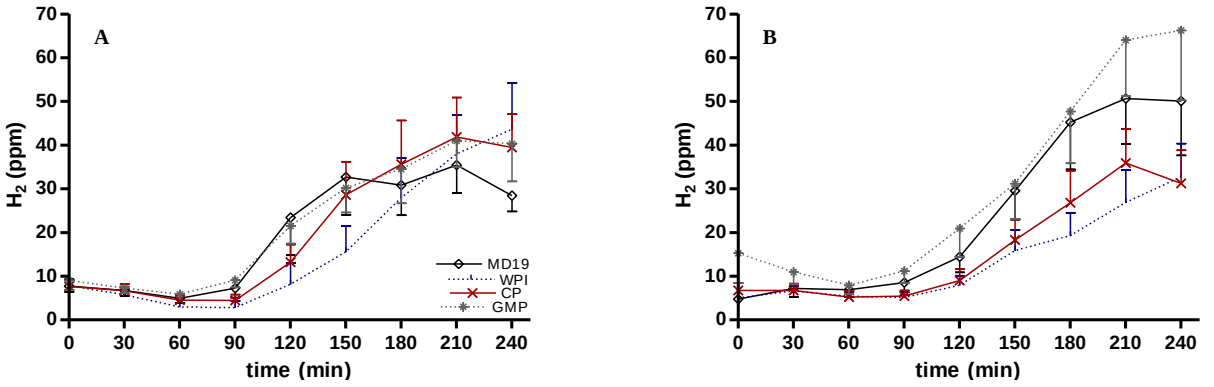


Figure 17: Hydrogen exhalation profiles reflecting oro-cecal transit time in the healthy group (**A**, n = 12-15) **and the prediabetic group** (**B**, n = 13-14). Transit time was determined by calculating of the inflexion point of H_2 over the time curve using the Boltzmann formula and significance was analyzed by One-way ANOVA with Tukey Kramer post hoc test. Significant differences in oro-cecal transit time were only observed in the control group: WPI drink compared to the CP drink, MD19 drink and the GMP drink ($P = 0.0014$, $P < 0.0001$, $P < 0.0001$, respectively).

Table 12: Oro-cecal transit time

Inflexion points	Control group	Prediabetic group
GMP	140±23 min[a]	175±25 min[a]
MD19	142±17 min[a]	158±21 min[a]
CP	151±24 min[a]	174±40 min[a]
WPI	179±27 min[b]	174±36 min[a]

Means with different letter codes are statistical different, $P < 0.05$.

4.4 Satiety effects of the test drinks

The results of the visual analogue scale (VAS) for the feeling of hunger demonstrate that the WPI drink induced the strongest suppression of the hunger feeling over the 240 min time course (**Figure 18 A**). The lowest levels were observed in healthy volunteers. There was no significant difference between the minimal scores of all test drinks. The feeling of hunger was significantly reduced after the WPI drink compared to the MD19 drink over the 240 min time course ($P = 0.0245$ for treatment x time effect with Tukey Kramer post hoc test, **Figure 18 A**). However, data of the feeling of hunger expressed in AUC did not demonstrate any significant difference between diets (**Figure 18 E**).

In prediabetic volunteers the feeling of hunger was only reduced after the CP drink which was significantly different to the MD19 and GMP drinks ($P = 0.0187$, $P = 0.0189$; treatment x time interaction with Tukey Kramer post hoc test (**Figure 18 B**). The AUC of the feeling of hunger ratings confirms the difference after the CP drink compared to the MD19 drink and the GMP drinks ($P = 0.0321$, $P = 0.0098$, respectively, **Figure 18 E**).

Plasma ghrelin concentrations also showed the strongest minima at 60 min after the GMP drink followed by the MD19, the WPI and the CP drinks in healthy volunteers. No significant differences could be detected between the test drinks (**Figure 18 C**). Peaks of the VAS and ghrelin are summarized in the **appendix Table 43**. Interestingly, a strong negative correlation was observed between glucagon and ghrelin after intake of the WPI and the CPI drinks in the healthy group ($r = -0.9265$, $P = 0.0009$, $R^2 = 0.8584$ **Figure 18 F** and $r = -0.8072$, $P = 0.0154$, $R^2 = 0.6516$, respectively). Ghrelin concentrations declined with the strongest peak at 90 min after the GMP drink followed by the CP drink with less pronounced changes in the prediabetic group. Peaks of the VAS and ghrelin are summarized in the **appendix Table 44**.

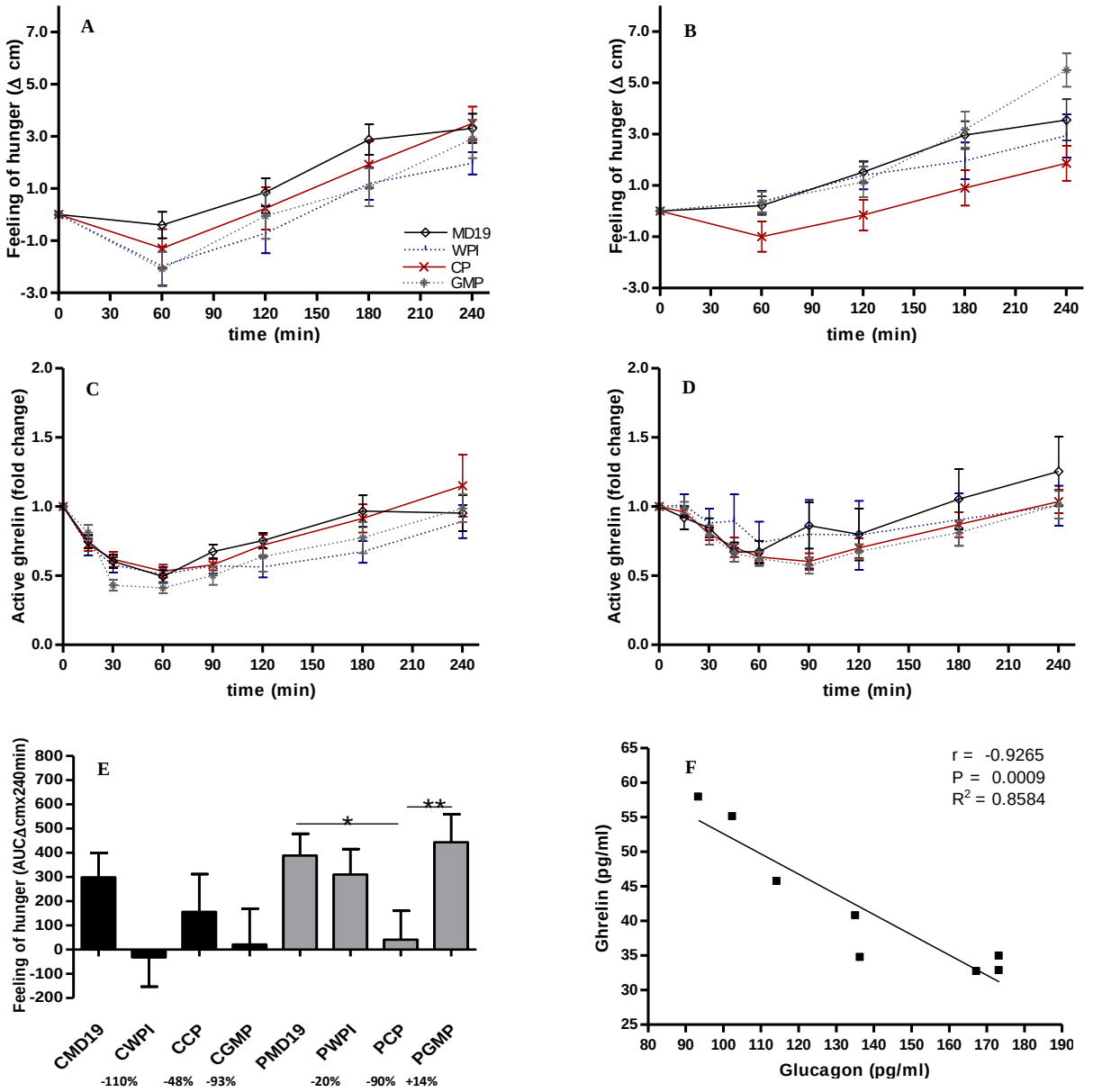


Figure 18: Time course of the hunger ratings and active plasma ghrelin levels and the subjective feeling of hunger after the various test drinks in the healthy group (**A**: n = 15, **C**: n = 9-11) and the prediabetic group (**B**: n = 15, **D**: n = 10-15). (**A**) In the control group, the feeling of hunger as determined by VAS was significantly different after the WPI drink compared to the MD19 test drink ($P = 0.0245$). (**B**) In the prediabetic group, the CP drink revealed the strongest decrease in the feeling of hunger compared to the MD19 test drink and the GMP drink ($P = 0.0187$, $P = 0.0189$, for all repeated-measures ANOVA treatment effects and treatment x time interactions with Tukey Kramer post hoc test). (**C, D**) No significant difference could be observed in ghrelin in both groups. (**E**) The feeling of hunger expressed in AUC over 240 min (repeated one way ANOVA with Tukey Kramer post hoc test). Significance: $_{***}P < 0.001$, $_{**}P < 0.01$, $_{*}P < 0.05$. (**F**) Correlation between glucagon and ghrelin after the WPI drink in the control group. C = control group, P = prediabetic group).

4.5 The tryptophan/long neutral amino acid ratio

Serotonin levels in brain are considered to participate in satiety control and tryptophan serves as a precursor of serotonin. Tryptophan levels are two fold higher in the whey protein isolate compared to the Na-caseinate whereas it is absent in GMP. As this allows to assess whether provision of Trp to brain changes satiety, we determined also the plasma Trp/LNAA ratio.The tryptophan/long neutral

amino acids plasma ratio (Trp/LNAA) ratio was significantly different between all test meals ($P < 0.0001$, for all) except between the WPI drink and the MD19 drink (**Figure 19 A**).

The AUC of the Trp/LNAA ratio was highest after the MD drink. It was reduced by 6% ($P < 0.0001$) after the WPI drink, by 34% after the CP drink ($P < 0.0001$) and by 63% ($P < 0.0001$) after the GMP drink when comparing it to the MD19 drink (**Figure 20**).

The Trp/LNAA ratio in the prediabeitc group differed significantly with the same pattern as in healthy volunteers. However, the AUC of the Trp/LNAA was significantly lower compared to the healthy group which may reflect insulin resistance. The Trp/LNAA ratio was different between all test meals ($P < 0.0001$, for all) except for the WPI and MD19 drinks (**Figure 19 B**). The AUC of the Trp/LNAA was highest after the MD19 drink. It was diminished by 9% after the WPI drink ($P = 0.0062$), by 35% after the CP drink ($P < 0.0001$) and by 68% ($P < 0.0001$) after the GMP drink compared to the MD19 drink (**Figure 20**).

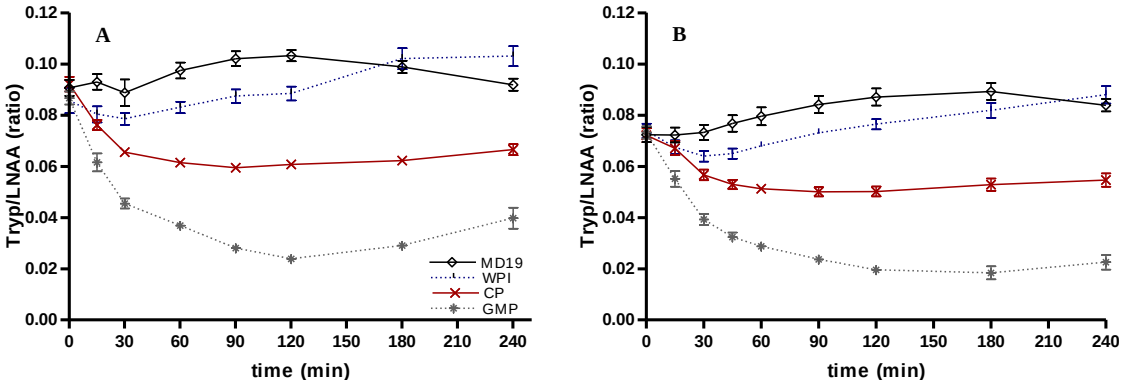


Figure 19: Time course of the plasma Trp/large neutral amino acid (LNAA) ratio derived from the plasma amino acid profiles from the healthy group (**A**, **C** n = 15) and the prediabetic group (**B**, **C** n = 15). (**A**) Significance for the Trp/LNAA was given in the healthy group when comparing the WPI drink to the CP drink and to the GMP drink ($P < 0.0001$ for each, respectively) and the CP drink compared to the MD19 drink and the GMP drink ($P < 0.0001$, for each). The Trp/LNAA ratio was also significantly different after the GMP drink compared to the MD19 drink ($P < 0.0001$). (**B**) In the prediabetic group, the Trp/LNAA ratio differs significantly in the same manner like in the control group. For A and B, significant differences were analyzed by repeated two way ANOVA and Tukey Kramer post hoc test. Comparison between groups was analyzed with T-test: For all diets: $P < 0.0001$. Significant differences in the AUC were determined by a repeated one way ANOVA with Tukey Kramer post hoc test. Significance: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. C = control group: n = 15, P = prediabetic group: n = 15.

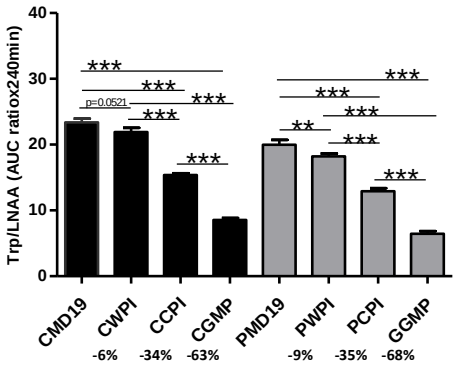


Figure 20: AUC of the Trp/LNAA ratio in healthy and prediabetic subjects.
Significant differences were determined by a repeated one way ANOVA with Tukey Kramer post hoc test.

4.6 Non-esterified fatty acid and β-hydroxybutyrate levels in plasma

The fold changes of plasma non esterified fatty acids (NEFAs) and plasma β-hydroxybutyrate (β-HB) after intake of the test drinks are illustrated in **Figure 21 A** and **C**. Concentrations of NEFAs decreased after all 4 test drinks in healthy volunteers. The AUC of NEFA levels over 240 min after the GMP drink was significantly reduced compared to the MD19 and CP drinks ($P = 0.0005$, $P = 0.0083$, respectively, **Figure 21 E**).

In the prediabetic group, the most pronounced decrease in NEFA concentrations was observed after 120 min for all test drinks. The minimum level of NEFAs was significantly different after the GMP drink compared to the MD19 drink ($P = 0.0469$). NEFA concentrations also differed significantly after the GMP drink compared to the WPI drink at time point 240 min ($P < 0.0001$, treatment x time effect with Tukey Kramer post hoc test, **Figure 21 B**). No differences of NEFAs were observed after all test drinks related to the AUC in the prediabetic group (**Figure 21 E**).

β-hydroxybutyrate levels were strongly decreased after the GMP and MD19 drinks in the healthy volunteers. In contrast, concentrations of β-HB did only change slightly after the WPI drink. Even an increase after intake of the CP drink with a peak at 60 min was observed which was significantly different to the minimum after the MD19 and GMP drinks ($P = 0.0045$, $P = 0.0177$, respectively, **Figure 21 C**). Peak levels of NEFAs and β-HB are summarized in the **appendix Table 43**. The AUC of β-HB after the CP drink was significantly higher to that of the GMP drink ($P = 0.0149$) over the 240 min time course (**Figure 21 F**). Interestingly, strong correlations could be observed between isoleucine and NEFAs, isoleucine and β-HB, glucagon and NEFAs after intake of the GMP drink as shown in **Figure 22**.

In prediabetics, β-HB decreased strongest after the GMP and MD19 drinks, respectively. Minima of β-HB concentrations after the GMP and the MD19 drinks were significantly lower to those reached with the WPI and CP drinks ($P = 0.0034$, $P = 0.0428$, $P < 0.0001$, $P = 0.0013$, respectively) (**Figure 21 D**). Peak levels of NEFAs and β-HB are summarized in the **appendix Table 44**. Also the AUC of β-HB showed the difference of MD19 compared to the WPI and CP drinks ($P < 0.05$ for each, **Figure 21 F**). Strong correlations were also observed in the prediabetic group between isoleucine and NEFAs ($r = -0.9542$, $P < 0.0001$, $R^2 = 0.9104$) and isoleucine and β-HB concentrations ($r = -0.9335$, $P = 0.0002$, $R^2 = 0.8713$).

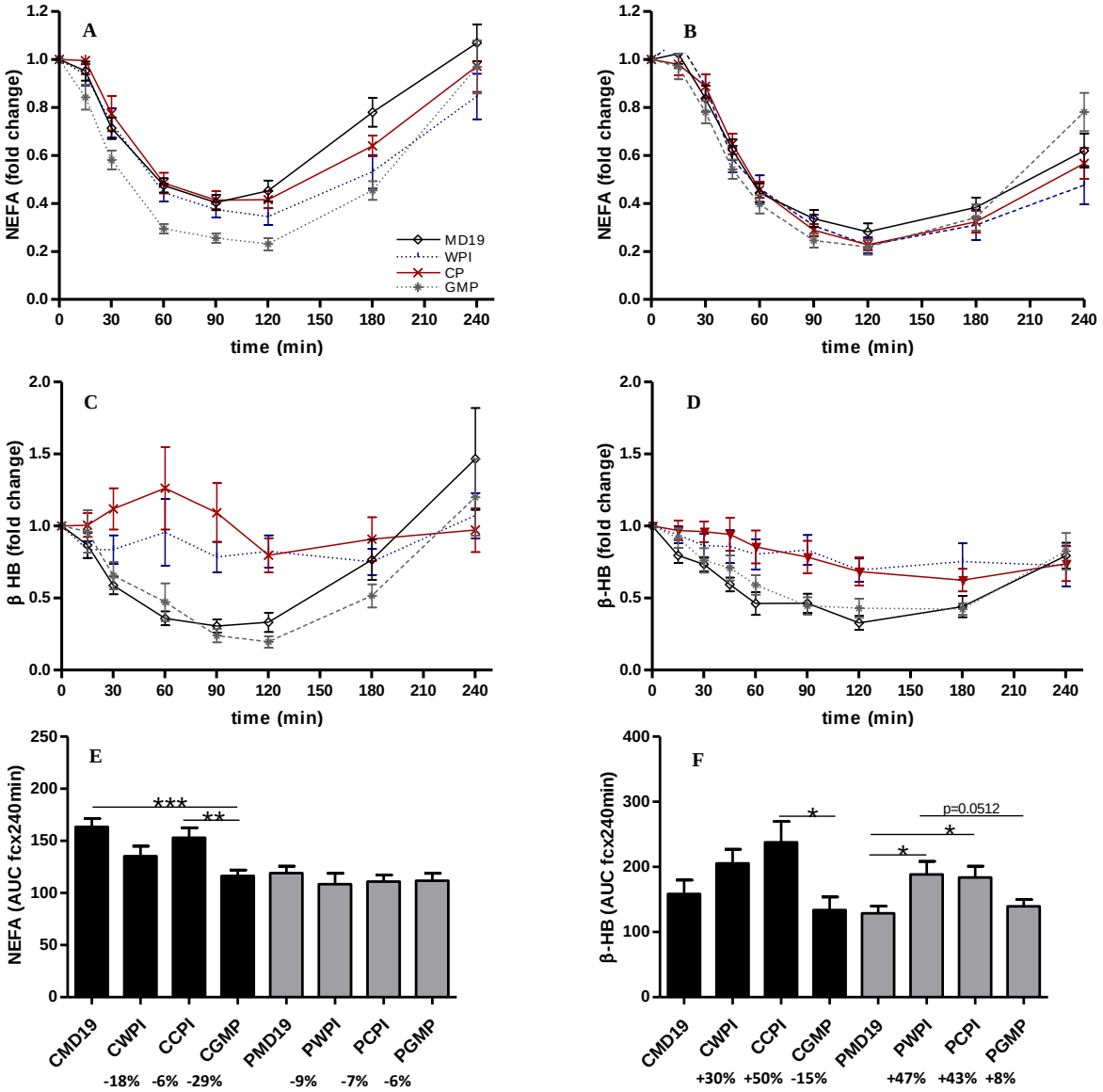


Figure 21: Plasma non esterified fatty acids and β-hydroxybutyrate concentrations over the 240 min time course in the healthy group (**A**, **C**: n = 15) and in the prediabetic group (**B**, **D**: n = 15). (**A**) NEFA levels were significantly different in the healthy group when comparing the CP drink and the MD19 drink to the GMP drink ($P = 0.0141$, $P = 0.0048$, respectively), which is also reflected by the AUC (**E**). In the prediabetic group no significant differences were observed between test drinks (**B**, **E**). β-HB was significantly different in the healthy group (**C**) when comparing the GMP drink with the CP drink ($P = 0.0268$). (**D**) It was also significantly different in the prediabetic group after the WPI drink and the CP drink compared to the MD19 drink ($P = 0.0117$, $P = 0.0106$, respectively). Significant differences between diets were analyzed by repeated two way ANOVA with treatment x time interactions and Tukey Kramer post hoc test. (**E, F**) AUC of NEFAs and β-HB was analyzed by repeated one way ANOVA followed by Tukey Kramer post hoc test. Significance: $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$.

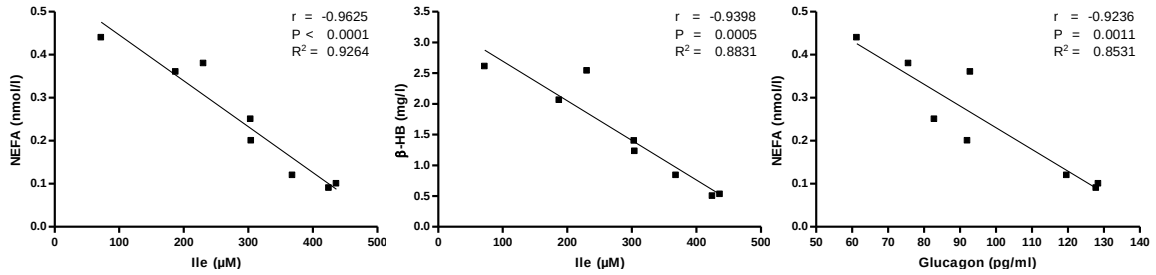


Figure 22: Correlations of Ile with NEFAs and β-HB and glucagon with NEFAs observed after intake of the GMP drink in the control group.

4.7 Postprandial plasma amino acid and metabolite changes

4.7.1 Correlations between amino acids contained in the test drinks and the plasma amino acid responses

For assessing differences in systemic amino acid levels caused by the test drinks and whether they relate to the ingested amount of the amino acids, the ratio of the amino acids contained in the milk proteins was calculated in relation to the maximal fold changes of plasma amino acids (**Figure 23**). The amino acids asparagine, aspartic acid, glutamic acid and methionine were excluded from the calculation but for the remaining amino acids strong correlations were observed for this relationship. This was not expected since according to literature up to 70% of the amino acid absorbed could be metabolized in the splanchnic bed (liver and gut) before they enter systemic circulation (Wu et al. 1998, Gelfand et al. 1986). This however may depend very much on the amount of protein deliverd to the gut which was with 50g rather high in the present study.

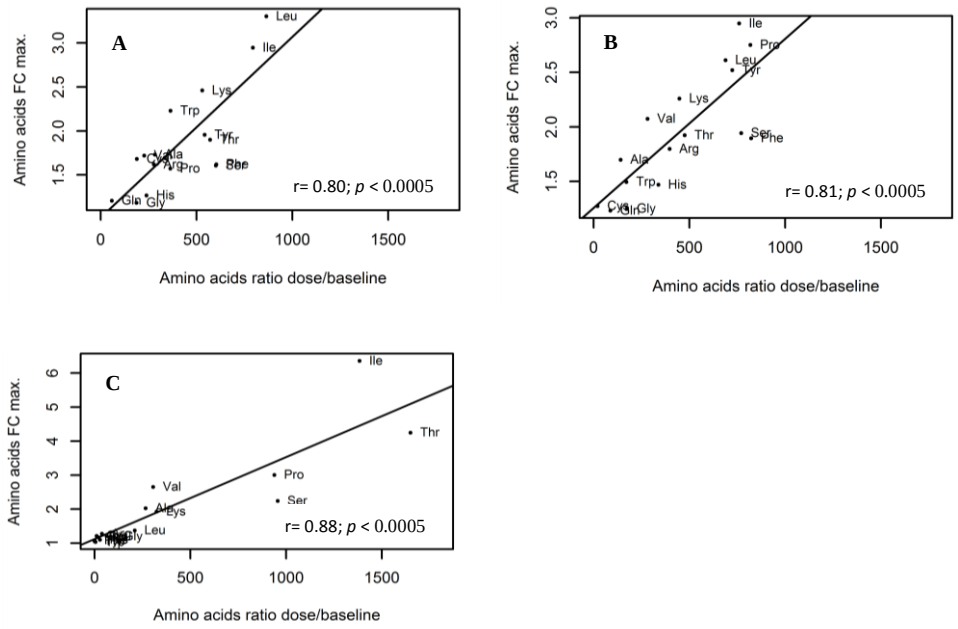


Figure 23: Kendall's tau correlation between the amino acid ratio of the dose ingested versus the plasma amino acid baseline to maximum fold-change ration of plasma amino acids within the 4 h test period in the healthy group. A = WPI drink, B = CP drink, C = GMP drink. The amino acids asparagine, aspartic acid, glutamic acid and methionine were excluded.

Also in the prediabetic group, strong correlations could be observed between the amount of amino acids ingested with the different proteins and plasma amino acid fold change over baseline. Asparagine, aspartic acid, glutamic acid and methionine were excluded from calculation (**Figure 24**).

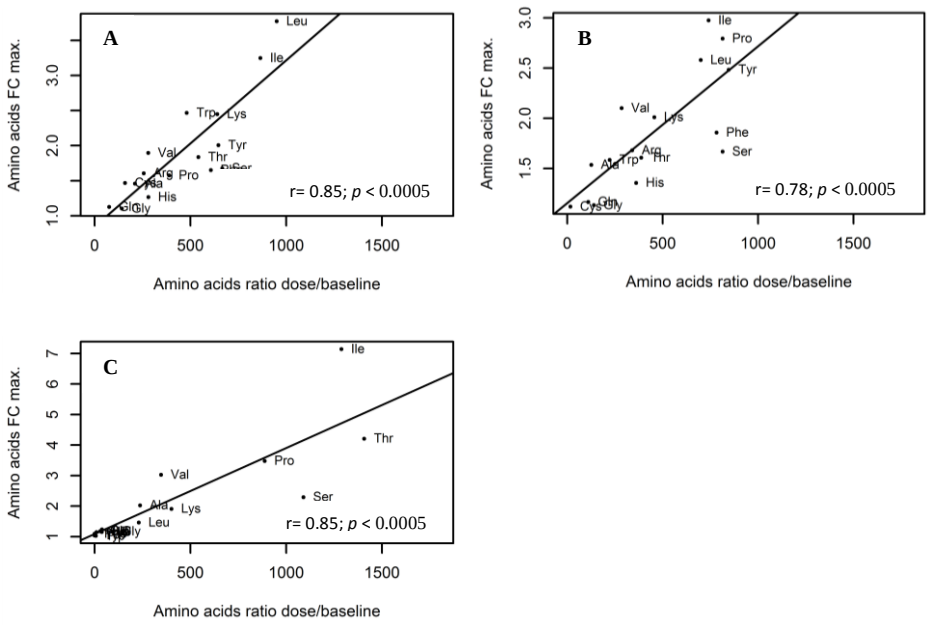


Figure 24: Kendall's tau correlation between the amino acid ratio of the dose ingested versus the maximum fold-change reached in plasma within the 4 h intervention in the prediabetic group. A = WPI drink, B = CP drink, C = GMP drink. The amino acids asparagine, aspartic acid, glutamic acid and methionine were excluded.

4.7.2 Postprandial plasma BCAA levels

Branched chain amino acids are the most abundant amino acids in milk proteins when compared to other food. As illustrated in **Figure 25**, the plasma BCAA levels increased corresponding to their content in the ingested proteins whereas levels decreased after the MD19 drink. Isoleucine concentration was extremely high after the GMP drink (6.13 ± 0.28 fold) which was significantly different to the peaks observed after the WPI and CP drinks and the minimum after MD19 ($P < 0.0001$, for all). In contrast, postprandial isoleucine excursions were not different after the WPI and CP drinks (**Figure 25 A**) with AUC values provided in **Figure 25 G**.

In the prediabetic group the high isoleucine peak (6.23 ± 0.62 fold) after GMP was also observed which was significantly different from that after the WPI ($P = 0.0044$) and CP drinks ($P = 0.0016$) with no significance between these two proteins (**Figure 25 D**). The AUC of isoleucine was also drastically elevated after the GMP drink (+576%), followed by the WPI and CP drinks (+253%, +211%, respectively) compared to MD19 (**Figure 25 G**).

Plasma leucine showed highest levels after the WPI drink (3.17 ± 0.14 fold) significantly different to the CP, GMP and MD19 drinks in healthy volunteers ($P < 0.0001$, for all) as shown in **Figure 25 B**. The WPI drink also elicited the highest AUC (+235%), followed by the CP drink (+155%) and the GMP drink (+37%) and these AUC values reflect the different amount presented in the protein (**Figure 25 H**).

The same pattern and significances were observed in the prediabetic group (**Figure 25 E**). The AUC of leucine was highest after the WPI drink (+291%), followed by the CP drink (+161%) and much less so after the GMP drink (+42%) when compared to MD19 (**Figure 25 H**).

GMP ingestion caused the highest valine peak which was significantly different compared to the WPI, CP and MD19 drinks in healthy volunteers ($P < 0.0001$, for all). A significant higher plasma valine peak could also be observed after the CP drink compared to the WP drink ($P = 0.0141$) and to the MD19 drink ($P < 0.0001$) (**Figure 25 C**). The AUC of valine was significantly higher after intake of the GMP drink than after the CP and WP drinks and compared to the MD19 drink (+150%, +98%, +64%, respectively) (**Figure 25 I**).
In the prediabetic group similar relationships are found as shown in **Figure 25 F** and **Figure 25 I**.

No differences in the branched chain amino acid profiles were observed between the WPI and CP drinks except for leucine. Total BCAA levels were highest after the GMP (+249%), followed by the WPI (+157%) and the CP drinks (+141%) when compared to MD19 (**Figure 25 J**) and a similar pattern is presented in the prediabetic group (GMP +252%, WPI +206% and CP +155%).

Amongst selected other plasma metabolites detected via the LC-MS/MS analysis, strong correlations were found between α-aminobutyrate (Abu) and valine, Abu and isoleucine, valine and isoleucine were observed after GMP intake. Abu can enter the pathway of BCAAs degradation at which it is further metabolized to propionyl coenzyme A by the branched chain ketoacid dehydrogenase or pyruvate dehydrogenase (Darling et al. 1999). BCAAs and aromatic amino acids (AAAs) share the same transporter (LAT1) for uptake into cells (Fernstrom 2005) and correspondingly, a strong correlation was observed between plasma BCAA and AAA levels after the WPI drink for example in the healthy group (**Figure 26**). Amino acid plasma peak levels are presented in the **appendix Table 40**.

In the prediabetic group strong correlations could also be observed between isoleucine and α-aminobutyrate ($r = 0.9710$, $P < 0.0001$, $R^2 = 0.9428$), valine and α-aminobutyrate ($r = 0.9204$, $P = 0.0004$, $R^2 = 0.8471$) as well as valine and isoleucine ($r = 0.9915$, $P < 0.0001$, $R^2 = 0.9831$) levels after the WPI drink. Isoleucine correlated also well with α-aminobutyrate after the CP drink ($r = 0.9635$, $P < 0.0001$, $R^2 = 0.9283$) but not with valine. In contrast to the healthy volunteers, no strong correlations could be found after the GMP drink between these amino acids and α-aminobutyrate levels. Peak levels of all plasma amino acids are presented in the **appendix Table 41**.

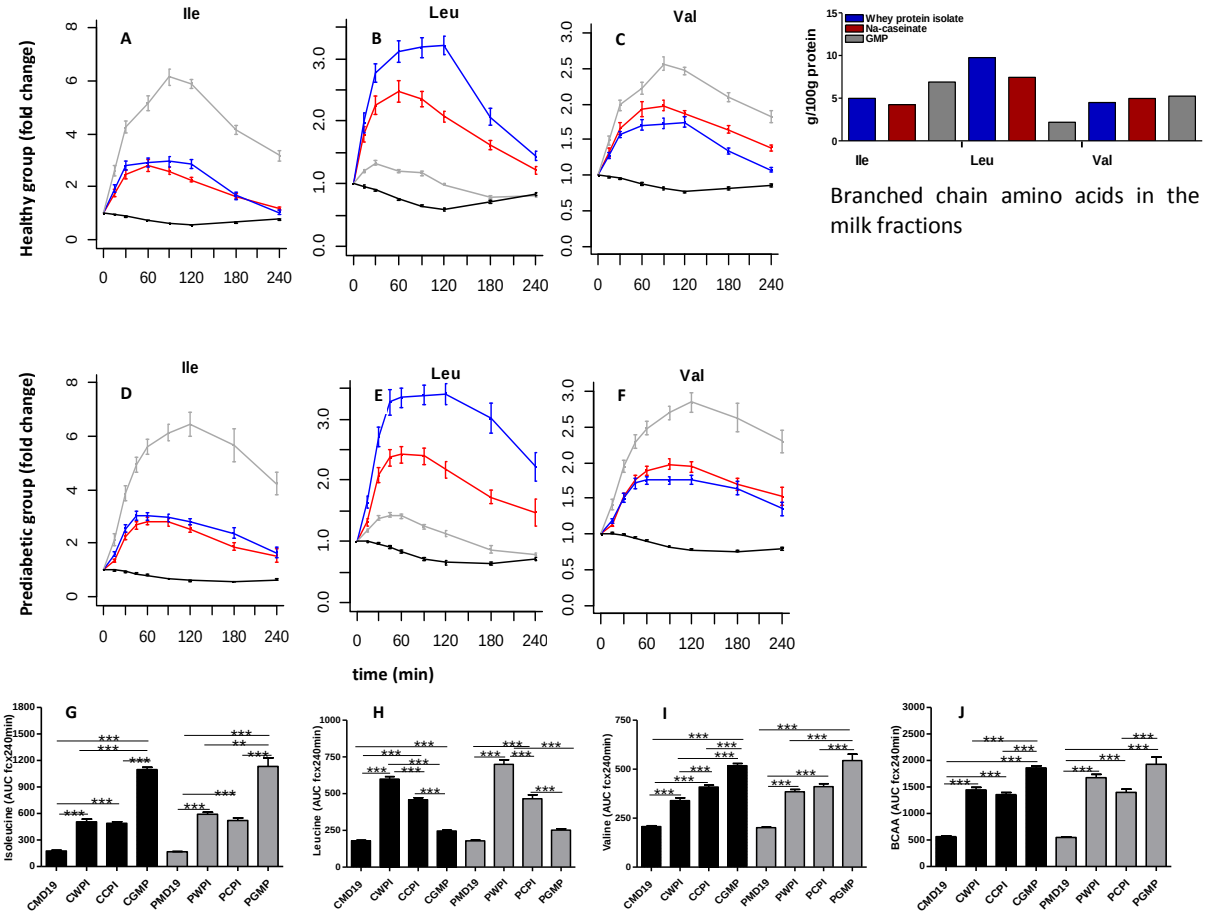


Figure 25: Time courses and AUC of fold-changes of plasma branched chain amino acids after intake of the WPI (blue), CP (red), GMP (grey) and MD19. (**A**) **Healthy group** (n = 15): **Isoleucine** was significantly different after the WPI drink compared to the GMP drink and the MD19 drink ($P < 0.0001$, for each). The CP drink also elicited a difference in isoleucine response compared to the GMP drink and the MD19 drink ($P < 0.0001$, for all). (**B**) **Leucine** was significantly different after the WPI drink compared to the CP drink, GMP drink and the MD19 drink ($P < 0.0001$, for each). A significant difference in plasma leucine was also observed after the CP drink compared to the GMP drink and the MD19 drink ($P < 0.0005$, for each). (**C**) **Valine** was significantly different after the WPI drink compared to the CP drink, the GMP drink and the MD19 drink ($P < 0.0001$, for all). The CP drink induced also a significant difference in valine compared to the GMP drink and the MD19 drink ($P < 0.0001$, for each). (**D, E, F**) The same pattern of significance could also be observed in the **prediabetic group** (n = 15). Significant differences between diets were determined by repeated-measures ANOVA treatment and Tukey Kramer post hoc test. Significant differences of the AUC of isoleucine, leucine, valine and total BCAA as shown in **G**, **H**, **I**, **J** were analysed by repeated one way ANOVA followed by Tukey Kramer post hoc test. Significance: $_{***}P < 0.001$, $_{**}P < 0.01$, $_{*}P < 0.05$. (C = control group, P = prediabetic group).

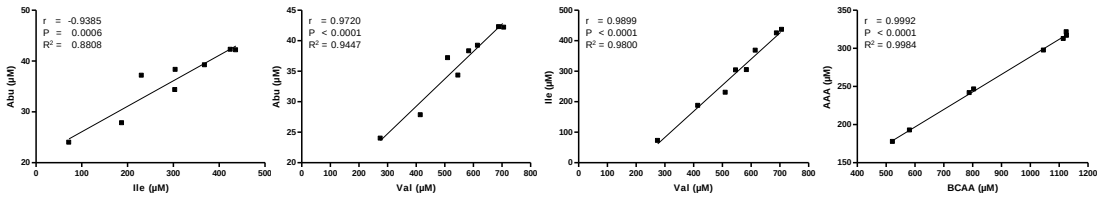


Figure 26: Pearson's correlations between plasma Ile and Abu, Val and Abu, valine and Ile levels after the GMP drink and between BCAAs and AAAs after the WPI drink in the healthy group.

4.7.3 Postprandial plasma BCAA levels in relation to insulin resistance

Insulin resistance is not only reflected by abnormal postprandial glucose and insulin excursions but becomes also visible by changes in amino acid profiles. **Figure 27** presents the comparison of the postprandial BCAA responses in healthy and prediabetic volunteers. A comparison of all amino acid

profiles between the two groups after each test drink is provided in the **appendix Figures 40 - 43**. After MD19, a strong decline of a large number of amino acids was found and amongst them also the BCAAs. Similar changes have been demonstrated after an oral glucose tolerance test (Skurk et al. 2001, Shaham et al. 2008).

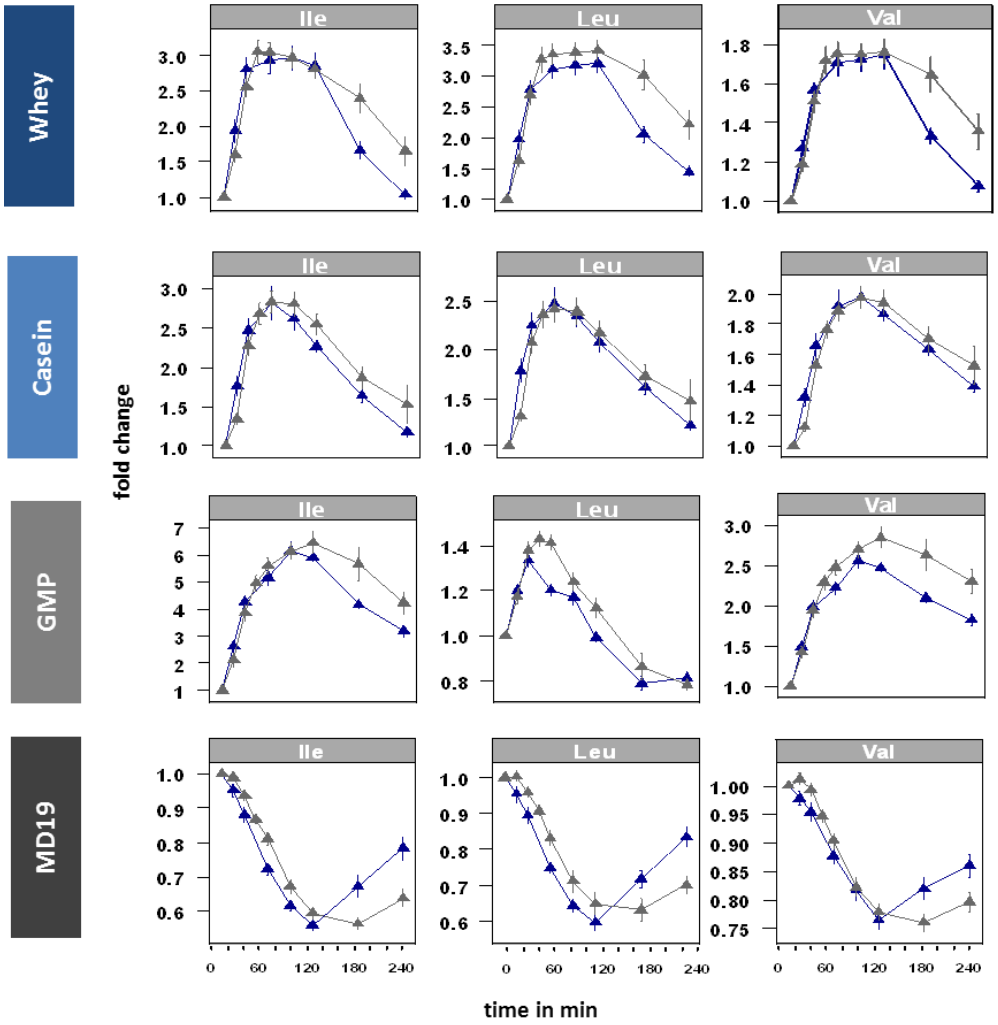


Figure 27: Postprandial BCAA response in healthy (blue) and prediabetic (grey) volunteers after ingestion of either the WPI drink, CP drink, GMP drink or MD19 drink.

A comparison of amino acid concentrations between groups at time points 180 min and 240 min was performed using Student's t-test because a delay of the amino acid response in the prediabetic group was observed especially towards the end of the test period. Differences in the plasma amino acids between groups are shown in **Tables 13** to **16**.

Table 13: Comparison of amino acid changes between groups at time points 180 min and 240 min after ingestion of the WPI drink

	Healthy	Prediabetic		Healthy	Prediabetic	
	fc 180 min	**fc 180 min**	**P value**	**fc 240 min**	**fc 240 min**	**P value**
Aad	5.28±0.46	7.87±0.83	**0.008**	3.26±0.38	6.99±0.99	**0.002**
Abu	0.94±0.05	1.15±0.05	**0.001**	0.83±0.04	1.07±0.05	**0.001**
Ala	1.34±0.06	1.19±0.07	**0.050**	1.22±0.05	1.06±0.04	**0.008**
Arg	1.08±0.06	1.2±0.04	**0.043**	0.9±0.03	0.96±0.04	0.116
Asn	1.19±0.04	1.19±0.05	0.478	0.99±0.03	1.01±0.05	0.342
Asp	1.65±0.23	2.48±0.31	**0.020**	1.01±0.13	2.25±0.57	**0.026**
Cit	1.41±0.06	1.54±0.07	0.097	1.21±0.05	1.52±0.07	**0.001**
Cys	1.3±0.05	1.21±0.05	0.108	1.03±0.03	1.11±0.03	**0.031**
EtN	0.89±0.09	0.96±0.02	0.237	0.91±0.11	0.97±0.04	0.314
Gln	1.03±0.03	1.01±0.03	0.371	1.04±0.03	0.96±0.03	**0.035**
Glu	1.3±0.11	1.63±0.15	**0.048**	0.93±0.11	1.32±0.11	**0.009**
Gly	0.85±0.03	0.8±0.02	0.073	0.82±0.03	0.77±0.04	0.131
His	1.01±0.03	1±0.02	0.446	0.96±0.02	0.93±0.03	0.222
Ile	1.56±0.13	2.39±0.2	**0.001**	0.98±0.07	1.66±0.2	**0.002**
Leu	2.07±0.14	3.01±0.24	**0.001**	1.46±0.09	2.22±0.24	**0.005**
Lys	1.57±0.09	1.87±0.12	**0.029**	1.25±0.06	1.4±0.09	0.083
Met	1.76±0.41	1.68±0.13	0.430	1.28±0.29	1.12±0.07	0.299
Orn	1.18±0.04	1.29±0.07	0.087	0.97±0.03	1.2±0.1	**0.019**
PEtN	0.97±0.05	0.89±0.1	0.215	1.04±0.06	0.89±0.04	**0.029**
Phe	1.21±0.04	1.24±0.06	**0.002**	0.84±0.03	0.99±0.06	**0.018**
Pro	1.21±0.14	1.32±0.06	0.072	1.05±0.04	1.15±0.04	**0.045**
Ser	1±0.04	0.97±0.03	0.280	0.85±0.03	0.92±0.11	0.273
Tau	0.87±0.02	0.81±0.02	**0.025**	0.94±0.03	0.81±0.02	**0.001**
Thr	1.35±0.06	1.47±0.08	0.116	1.13±0.05	1.27±0.07	**0.050**
Trp	1.79±0.1	2.23±0.11	**0.004**	1.38±0.09	1.82±0.11	**0.002**
Tyr	1.4±0.06	1.72±0.11	**0.011**	1.14±0.05	1.45±0.11	**0.011**
Val	1.27±0.06	1.64±0.09	**0.001**	1.03±0.04	1.36±0.09	**0.002**

Table 14: Comparison of amino acid changes between groups at time points 180 min and 240 min after ingestion of the CP drink

	Healthy	Prediabetic		Healthy	Prediabetic	
	fc 180 min	**fc 180 min**	**P value**	**fc 240 min**	**fc 240 min**	**P value**
Aad	2.63±0.19	4.13±0.65	**0.024**	1.86±0.14	3.15±0.43	**0.007**
Abu	1.01±0.04	1.06±0.05	0.214	0.95±0.03	1.04±0.05	0.085
Ala	1.22±0.06	1.19±0.06	0.377	1.11±0.04	1.14±0.04	0.326
Arg	1.2±0.04	1.19±0.04	0.406	0.99±0.03	1.06±0.07	0.209
Asn	1.29±0.05	1.1±0.03	**0.001**	1.09±0.03	1±0.05	0.086
Asp	1.31±0.12	1.46±0.28	0.309	1.05±0.13	1.15±0.26	0.369
Cit	1.6±0.08	1.41±0.06	**0.037**	1.34±0.06	1.29±0.08	0.304
Cys	0.96±0.05	0.9±0.03	0.155	0.95±0.07	0.88±0.03	0.175
EtN	0.53±0.54	0.96±0.03	0.223	0.81±0.25	1.03±0.04	0.207
Gln	1.06±0.03	0.96±0.02	**0.003**	1.05±0.02	0.95±0.03	**0.005**
Glu	1.18±0.11	1.44±0.16	0.095	0.81±0.10	1.01±0.12	0.100
Gly	0.92±0.02	0.84±0.02	**0.004**	0.91±0.02	0.83±0.02	**0.003**
His	1.09±0.03	0.95±0.02	**0.001**	1.00±0.03	0.94±0.05	0.133
Ile	1.66±0.09	1.87±0.15	0.126	1.18±0.07	1.53±0.25	0.101
Leu	1.63±0.07	1.73±0.12	0.251	1.22±0.05	1.47±0.22	0.141
Lys	1.46±0.06	1.33±0.06	0.074	1.19±0.04	1.22±0.12	0.396
Met	1.70±0.14	1.69±0.1	0.466	1.32±0.14	1.68±0.2	0.075
Orn	1.40±0.05	1.4±0.06	0.482	1.17±0.05	1.21±0.08	0.300
PEtN	1.13±0.06	0.99±0.05	**0.038**	1.20±0.11	1.09±0.07	0.215
Phe	1.27±0.04	1.39±0.05	**0.042**	1.05±0.03	1.16±0.08	0.095
Pro	1.97±0.14	2.22±0.17	0.129	1.62±0.09	1.9±0.17	0.080
Ser	1.20±0.04	1.06±0.04	**0.007**	1.05±0.03	0.93±0.04	**0.009**
Tau	0.92±0.03	0.82±0.02	**0.006**	0.96±0.02	0.87±0.04	**0.023**
Thr	1.33±0.08	1.19±0.04	0.054	1.17±0.06	1.07±0.06	0.115
Trp	1.11±0.03	1.24±0.05	**0.012**	0.94±0.03	1.09±0.07	**0.032**
Tyr	1.89±0.08	1.98±0.11	0.237	1.5±0.05	1.78±0.15	0.052
Val	1.66±0.05	1.7±0.08	0.329	1.4±0.04	1.53±0.13	0.178

Table 15: Comparison of amino acid changes between groups at time points 180 min and 240 min after ingestion of the GMP drink

	Healthy	Prediabetic		Healthy	Prediabetic	
	fc 180 min	**fc 180 min**	**P value**	**fc 240 min**	**fc 240 min**	**P value**
Aad	1.51±0.14	3.32±0.36	**0.0001**	1.21±0.11	2.4±0.35	**0.003**
Abu	1.61±0.05	1.98±0.15	**0.014**	1.56±0.07	1.98±0.16	**0.013**
Ala	1.41±0.07	1.56±0.11	0.112	1.23±0.06	1.35±0.08	0.113
Arg	0.96±0.03	0.94±0.02	0.378	0.87±0.03	0.86±0.02	0.495
Asn	1.37±0.06	1.57±0.13	0.091	1.18±0.03	1.2±0.06	0.391
Asp	1.26±0.13	1.94±0.34	**0.042**	0.96±0.16	1.29±0.15	0.072
Cit	1.21±0.04	1.16±0.08	0.285	1.07±0.03	1.13±0.07	0.193
Cys	0.91±0.03	1.02±0.04	**0.019**	0.87±0.04	0.99±0.04	**0.018**
EtN	1.19±0.24	0.94±0.05	0.157	1.18±0.19	0.94±0.05	0.121
Gln	1.00±0.02	1.94±0.34	0.117	1.01±0.02	1.29±0.15	0.424
Glu	1.3±0.17	1.75±0.25	0.077	0.88±0.1	1.04±0.08	0.102
Gly	0.93±0.03	0.95±0.03	0.325	0.92±0.03	0.92±0.03	0.487
His	0.93±0.02	0.9±0.02	0.171	0.95±0.02	0.96±0.05	0.401
Ile	4.23±0.15	5.04±0.62	0.112	3.21±0.18	3.79±0.41	0.103
Leu	0.79±0.02	0.81±0.05	0.387	0.81±0.03	0.77±0.02	0.157
Lys	1.23±0.04	1.37±0.1	0.111	1.06±0.03	1.11±0.05	0.196
Met	1.20±0.10	1.48±0.1	**0.037**	1.17±0.11	1.22±0.09	0.348
Orn	1.1±0.060	1.12±0.05	0.403	0.99±0.05	1.06±0.05	0.166
PEtN	1.08±0.06	1.21±0.07	0.092	1.05±0.04	1.07±0.08	0.408
Phe	0.56±0.03	0.51±0.02	0.067	0.66±0.03	0.57±0.04	0.051
Pro	1.92±0.07	2.59±0.28	**0.018**	1.55±0.07	1.96±0.17	**0.020**
Ser	1.28±0.05	1.45±0.11	0.090	1.14±0.04	1.24±0.06	0.105
Tau	0.95±0.02	0.97±0.02	0.287	0.92±0.03	0.92±0.03	0.482
Thr	2.68±0.11	3.12±0.27	0.074	2.29±0.11	2.55±0.18	0.116
Trp	0.57±0.03	0.51±0.03	0.076	0.66±0.04	0.54±0.04	**0.019**
Tyr	0.54±0.02	0.53±0.02	0.266	0.58±0.04	0.53±0.03	0.157
Val	2.14±0.06	2.39±0.2	0.118	1.87±0.07	2.10±0.15	0.086

Table 16: Comparison of amino acid changes between groups at time points 180 min and 240 min after ingestions of the MD19 drink

	Healthy	Prediabetic		Healthy	Prediabetic	
	fc 180 min	**fc 180 min**	**P value**	**fc 240 min**	**fc 240 min**	**P value**
Aad	1.00±0.08	0.83±0.08	0.076	0.89±0.07	0.87±0.07	0.399
Abu	0.83±0.02	0.76±0.01	**0.006**	0.85±0.02	0.76±0.02	**0.001**
Ala	1.01±0.05	1.01±0.04	0.472	0.98±0.06	1.00±0.03	0.402
Arg	0.84±0.02	0.77±0.02	**0.008**	0.9±0.03	0.81±0.02	**0.009**
Asn	0.95±0.03	0.83±0.02	**0.003**	0.98±0.04	0.86±0.02	**0.004**
Asp	0.94±0.13	1.13±0.24	0.249	0.95±0.1	1.29±0.31	0.152
Cit	0.80±0.02	0.70±0.03	**0.004**	0.85±0.03	0.77±0.03	**0.036**
Cys	0.96±0.07	0.96±0.03	0.499	0.98±0.08	0.94±0.03	0.295
EtN	0.98±0.09	1.05±0.04	0.225	1.02±0.11	1.08±0.04	0.304
Gln	1.02±0.03	0.96±0.03	0.054	1.06±0.03	0.99±0.02	**0.022**
Glu	0.88±0.10	0.81±0.09	0.306	0.72±0.09	0.72±0.08	0.499
Gly	0.98±0.02	0.97±0.02	0.434	0.98±0.03	0.99±0.02	0.316
His	0.99±0.03	0.94±0.02	0.066	1.02±0.03	0.99±0.02	0.265
Ile	0.66±0.03	0.56±0.02	**0.004**	0.77±0.03	0.64±0.03	**0.002**
Leu	0.71±0.02	0.63±0.03	**0.026**	0.82±0.03	0.70±0.02	**0.001**
Lys	0.94±0.02	0.90±0.03	0.148	0.96±0.03	0.94±0.03	0.310
Met	0.85±0.15	0.70±0.06	0.181	0.98±0.16	0.83±0.05	0.179
Orn	0.82±0.03	0.79±0.02	0.172	0.80±0.02	0.80±0.04	0.494
PEtN	0.86±0.04	0.93±0.05	0.115	0.93±0.06	1.03±0.07	0.122
Phe	0.80±0.02	0.77±0.02	0.153	0.88±0.03	0.85±0.02	0.212
Pro	0.90±0.04	0.81±0.02	**0.035**	0.91±0.04	0.81±0.02	**0.007**
Ser	0.87±0.02	0.85±0.03	0.357	0.89±0.02	0.92±0.04	0.241
Tau	0.91±0.02	0.88±0.02	0.086	0.9±0.02	0.89±0.01	0.331
Thr	0.86±0.02	0.79±0.03	**0.038**	0.91±0.04	0.83±0.02	**0.047**
Trp	0.84±0.02	0.86±0.02	0.208	0.85±0.02	0.88±0.02	0.133
Tyr	0.75±0.02	0.69±0.02	**0.049**	0.8±0.03	0.77±0.02	0.192
Val	0.82±0.02	0.76±0.02	**0.012**	0.86±0.02	0.80±0.02	**0.015**

4.7.4 Postprandial plasma aromatic amino acid levels

GMP - which does not contain any aromatic amino acids - produced a noticeable difference in the postprandial amino acid profile as illustrated in **Figure 28**. A conspicuous decline in plasma tyrosine, phenylalanine and tryptophan could be observed after the GMP challenge even below levels observed after MD19 while the aromatic amino acids showed a marked increase after the CP and WPI drinks. The plasma minimum of tyrosine was reached 180 min after GMP (0.54 ± 0.02 fold) and that differed significantly to MD19 with lowest levels at 120 min (0.71 ± 0.10, $P < 0.0001$) as shown in **Figure 28 A**. The AUC of tyrosine increased by 156% after the CP drink and by 101% after the WPI drink. GMP reduced plasma tyrosine concentrations to less than those reached after MD19 (**Figure 28 I**).

Phenylalanine decreased most strongly after GMP at 180 min (0.56 ± 0.03 fold) compared to the minimum oberserved after MD19 at 120 min (0.75 ± 0.03 fold, $P < 0.0001$) as shown in **Figure 28 B**. The AUC of phenylalanine was elevated by 78% after the CP, by 53% after WPI and decreased by 10% after the GMP drink compared to the MD19 (**Figure 28 J**). Tryptophan levels also decreased after the GMP drink with a minimum level at 120 min (0.51 ± 0.03 fold) observed at 120 min as shown in **Figure 28 C**. The WPI drink induced the highest increase in the AUC of tryptophan (115%) In contrast, the AUC of tryptophan was diminished by 16% after the GMP drink when compared to the MD19 drink (**Figure 28 K**).

Interestingly, strong correlations could be observed between phenylalanine and Abu levels after the the WPI (**Figure 28 D**) and the CP drinks ($r = 0.9522$, $P = 0.0003$, $R^2 = 0.9066$).

Also in the prediabetic group the decline of plasma tyrosine, phenylalanine and tryptophan was larger after GMP than after MD19. The plasma minimum of tyrosine was reached 240 min after GMP (0.50 ± 0.03) and that differed significantly to MD19 with lowest levels at 180 min (0.69 ± 0.02, $P < 0.0001$) as shown in **Figure 28 E**. The AUC of tyrosine increased by 153% after the CP drink and by 109% after the WPI drink compared to the MD 19. In contrast, the GMP drink led to stronger reduction in tyrosine than after MD19 (-8%) as shown in **Figure 28 I**.

Phenylalanine decreased most strongly after GMP at 180 min (0.50 ± 0.02 fold) compared to the minimum oberserved after MD19 at 120 min (0.76 ± 0.01 fold, $P < 0.0001$) as shown in **Figure 28 F**. with the AUC data shown in **Figure 28 J**.

Tryptophan decreased as well after GMP (**Figure 28 G**). Taken together, all aromatic amino acids were reduced after the GMP drink more markedly in both, peak concentrations and AUC values than after the MD19 alone (**Figure 28 L**).

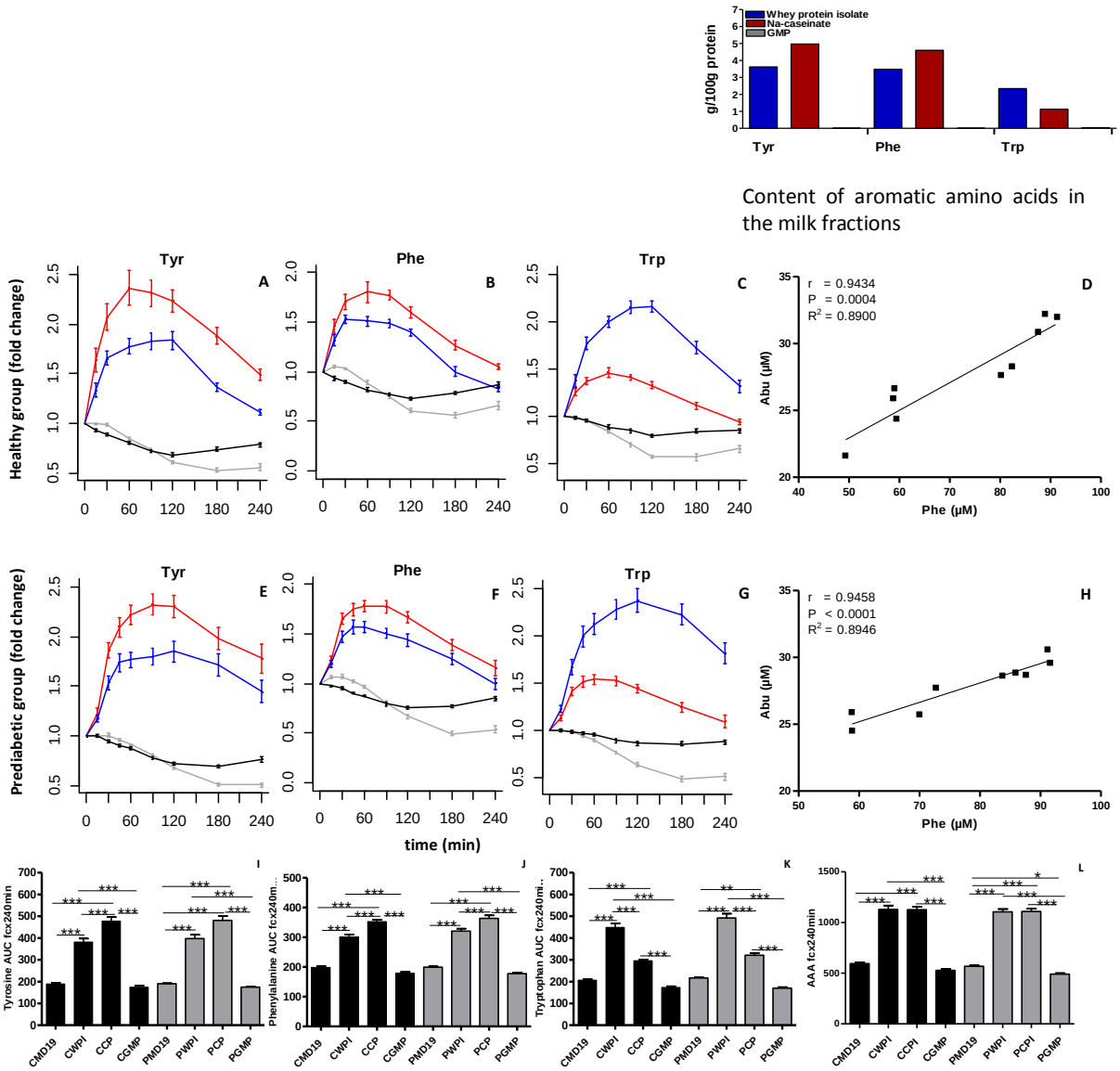


Figure 28: Time courses and AUC of plasma aromatic amino acids (fold change) **after intake of the WPI** (blue), **CP** (red), **GMP** (grey) **and MD19**. **Healthy group** (n = 15): (**A**) **Tyrosine** was significantly different after the WPI drink compared to the CP drink (P = 0.0011), GMP drink and the MD19 drink (P < 0.0001, for both). The CP drink induced also a significant difference in tyrosine compared to the GMP drink and the MD19 drink (P < 0.0001, for each) (**B**) **Phenylalanine** was significantly different after the WPI drink compared to the CP drink (P = 0.010), GMP drink and the MD19 drink (P < 0.0001, for each). A significant difference in plasma phenylalanine was also observed after the CP drink compared to the GMP drink and the MD19 drink (P < 0.0001, for each). (**C**) **Tryptophan** was significantly different after the WPI drink compared to the CP drink, GMP drink and the MD19 drink (P < 0.0001, for each). The CP drink also elicited a difference in tryptophan compared to the GMP drink and the MD19 drink (P < 0.0001, for all). The same pattern of significance could also be observed in the **prediabetic group (E, F, G)** (n = 15). Significance was determined by repeated-measures ANOVA treatment effects and treatment x time interactions with Tukey Kramer post hoc test. Significance of the AUC was analysed by repeated one way ANOVA followed by Tukey Kramer. Significance: *** P < 0.001, ** P < 0.01, * P < 0.05. (C = control group, P = prediabetic group). Strong correlations could be observed between phenylalanine and α-aminobutyrate after the WPI drink in both groups (**D, H**).

4.7.5 Postprandial plasma levels of threonine, α-aminobutyrate, tryptophan and α-aminoadipic acid

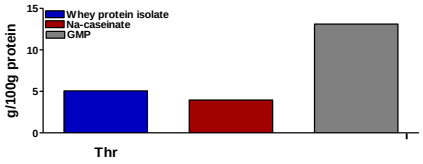
The time courses of plasma threonine levels are illustrated in **Figure 29.** Threonine increased after all 3 test drinks with highest levels at 90 min after the GMP (4.13 ± 0.21 fold) which was significantly different to the peaks obtained after the WPI and CP drinks ($P < 0.0001$, for both) (**Figure 29 A**). Also the AUC of threonine was significantly higher by 214% after the GMP compared to the MD19 drink ($P < 0.0001$) and 100% higher compared to those caused by WPI and CP drinks ($P < 0.0001$, for both) as shown in **Figure 29 I**. Levels of α-aminobutyrate (Abu) which is a degradation product of threonine also increased and peaked 90 min after GMP (1.99 ± 0.10 fold) and this was significantly different from WPI and CP (1.24 ± 0.05 fold, 60 min, 1.29 ± 0.05 fold, 60 min; $P < 0.0001$, for both) as shown in **Figure 29 B**. In case of GMP drink, the AUC increased by 80% when compared to MD19 ($P < 0.0001$) and was also significantly higher compared to the WPI and the CP drinks (+51%, +42%, $P < 0.0001$, for both) (**Figure 29 J**). Besides the strong correlation observed between threonine and Abu in case of GMP (**Figure 30 A**), strong correlations were also observed after the WPI and CP drinks (r = 0.8019, $P = 0.0167$, $R^2 = 0.6430$; r = 0.902, $P = 0.0012$, $R^2 = 0.8467$, respectively).

In the prediabetic group, threonine peaks were also highest after GMP (3.77 ± 0.25 fold) (**Figure 29 E**) and significantly different from that after the WPI and CP drinks ($P < 0.0001$, for both). Corresponding differences were found for the AUC values (**Figure 29 I**).
Levels of α-aminobutyrate also changed as in the healthy volunteers (**Figure 29 F**) with strong correlation between threonine and α-aminobutyrate changes (**Figure 29 B**).

α-Aminoadipic acid (Aad) is a degradation product of lysine but can additionally derived from tryptophan metabolism. In the healthy volunteers, the peak of Aad after the WPI (5.29 ± 0.50 fold) was significantly different to the peak of CP and GMP (2.97 ±0.21 fold, 1.77 ±0.14 fold; $P < 0.0001$, for both). The peak of Aad was also significantly higher after the CP drink compared to the GMP drink ($P = 0.0254$) (**Figure 29 D**). The AUC of α-aminoadipic acid increased strongest after the WPI drink compared to MD19, CP and GMP (313%, 68%, 170%, $P < 0.0001$, respectively) (**Figure 29 L**). No correlation could be found between lysine and Aad levels. However, a correlation could be observed after the GMP challenge between tryptophan and α-aminoadipic acid (r = -0.8138, $P = 0.0140$, $R^2 = 0.6622$).

In the prediabetic group, α-aminoadipic acid (Aad) increased higher after the WPI drink (7.43 ± 0.83 fold) than in healthy volunteers and peak levels were significantly different from those after CP and GMP (3.78 ± 0.69 fold, 3.38 ± 0.36 fold; $P = 0.0039$, $P = 0.0005$, respectively) as shown in **Figure 29 H** with similar changes in AUC values (**Figure 29 L**). No correlation was found between lysine and Aad levels either. However, also a high correlation could also be observed between

tryptophan and α-aminoadipic acid after the GMP challenge in the prediabetic group which is illustrated in **Figure 30 C**.



Threonine content in the milk fractions

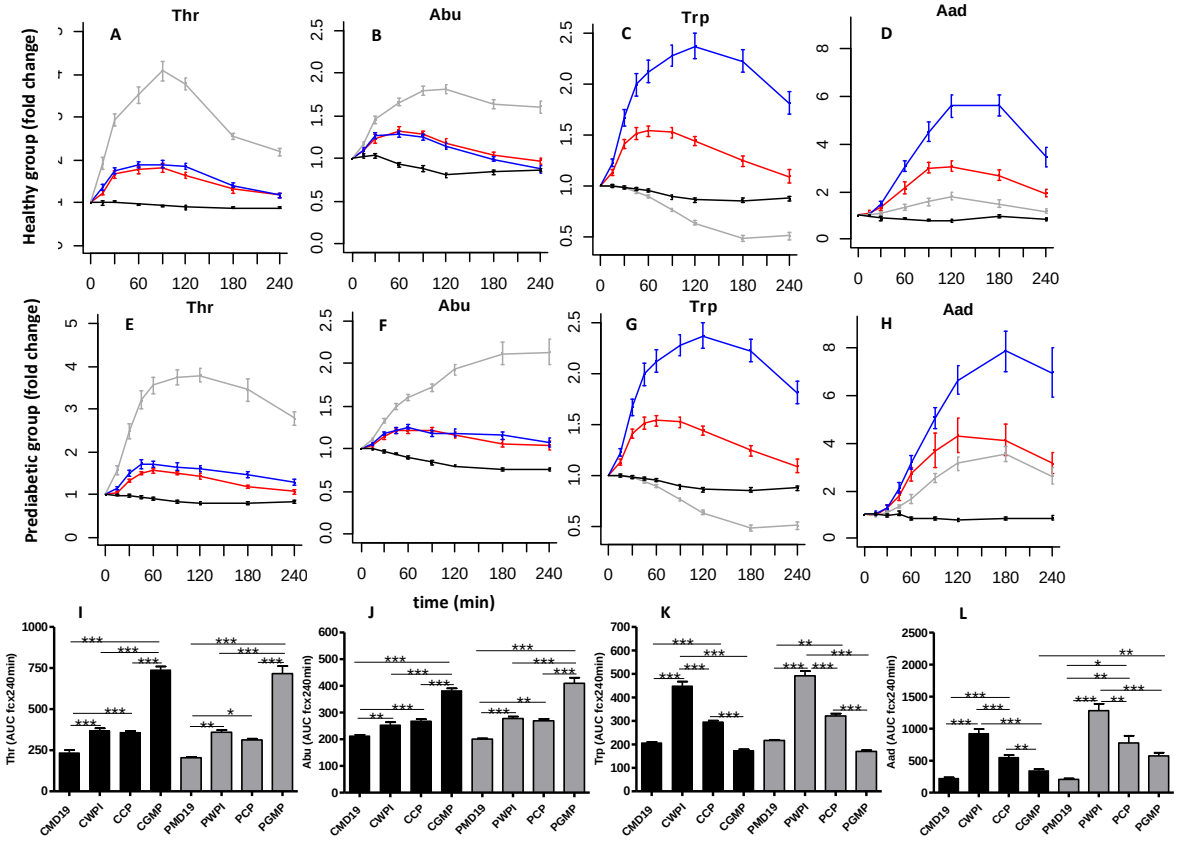


Figure 29: Time course and AUC of plasma threonine, α-aminobutyrate and tryptophan and α-aminoadipic acid after ingestion of either of the WPI (blue)**, CP** (red), **GMP** (grey**) and MD19 in the healthy and prediabetic group**. (**A, E**) Threonine was strongly elevated after the GMP drink which was sicnificantly different to that after the WPI drink, CP drink and the MD19 drink ($P < 0.0001$, for all). The WPI drink and the CP drink induced also a higher level of threonine compared to the MD 19 drink ($P = 0.0019$, $P = 0.0059$, respectively). (**B, F**) **Abu** was significantly different after the GMP drink compared to the WPI drink, CP drink and MD19 drink ($P < 0.0001$, for both). The WPI drink and the CP drink elicited also higher plasma Abu compared to the MD19 drink ($P < 0.0001$, for both). (**C, G**) **Tryptophan** was significantly different after the WPI drink compared to the CP drink, GMP drink and the MD19 drink ($P < 0.0001$, for each). The CP drink also elicited a difference in tryptophan compared to the GMP drink and the MD19 drink ($P < 0.0001$, for all). The same pattern of significance could also be observed in the prediabetic group (n = 15). (**D, H**) **Aad** was significantly different after the WPI drink compared to the CP drink ($P = 0.0003$), GMP drink and the MD 19 drink ($P < 0.0001$, for both). The CP drink induced also a significant difference in Aad compared to the GMP drink and the MD19 drink ($P < 0.0001$, for both). The same pattern of significance could also be observed in the **prediabetic group** (n = 15, Aad n = 14). Significance was determined by repeated-measures ANOVA treatment effects and treatment x time interactions followed by Tukey Kramer post hoc test. (**I, J, K, L**) AUC of threonine, Abu, tryptophan and Aad. AUC was analysed by one way ANOVA with Tukey Kramer. Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. (C = control group, P = prediabetic group).

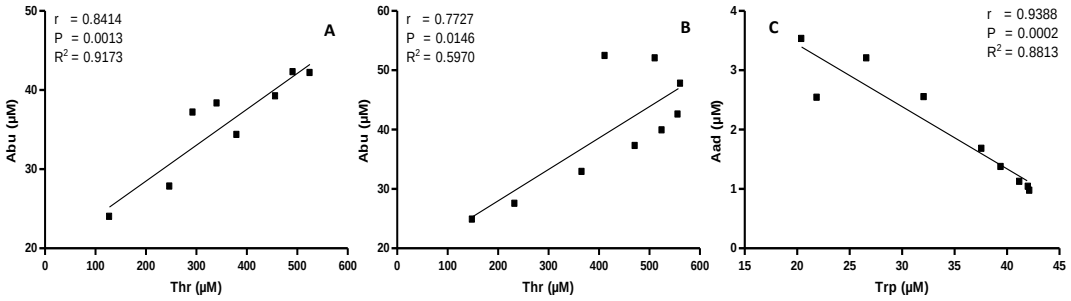


Figure 30: **Positive Pearson's correlations between threonine and α-aminobutyrate after the GMP challenge in healthy** (A) **and prediabetic volunteers** (**B**) **and a strong negative correlation between tryptophan and α-amino-adipic acid** was found in prediabetic volunteers after the GMP challenge (**C**).

4.7.6 Postprandial changes in plasma proline and ornithine levels

Ornithine is an intermediate of the urea cycle in liver and can also be derived from proline as illustrated in **Figure 31**.

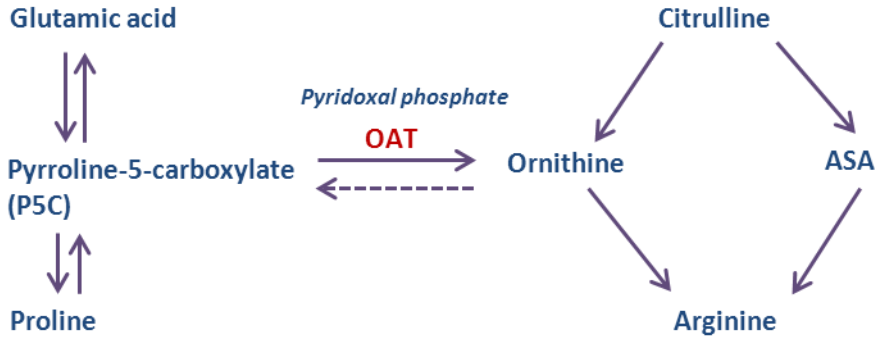


Figure 31: Metabolism of proline to ornithine (adapted from de Sain-van der Velden 2011).
OAT: Ornithine- δ-aminotransferase, ASA: arginiosuccinic acid

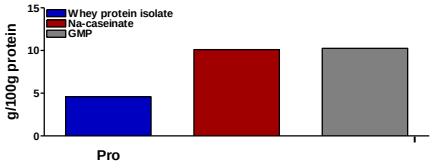
Proline and ornithine concentrations increased in plasma after the ingestion of all 3 test drinks with no change after MD19 intake. The time courses of proline and ornithine are shown in **Figure 32 A** and **B**. Peaks of proline were significantly higher after GMP (2.92 ± 0.15 fold) and CP drinks (2.63 ± 0.16 fold) compared to the WPI drink (1.5 ± 0.07 fold) and MD19 ($P < 0.0001$, for both). Interestingly, despite similar proline increases that crossly reflect the amount of proline in proteins ingested, the peak of ornithine was significantly higher after consumption of the CP drink when compared to the GMP drink (1.42 ± 0.06 fold, $P = 0.0143$) (**Figure 32 B**). AUC changes followed as shown in **Figure 32 G**.

The AUC of ornithine increased more after the CP drink than after the WPI and the GMP drinks. All diets induced significant differences among themselves in the AUC of ornithine ($P < 0.0020$, for all) except between the WPI drink and the GMP drink (**Figure 32 H**).

A strong correlation between proline and ornithine after intake of the CP and WPI drinks was observed ($r = 0.97$, $P < 0.0001$, $R^2 = 0.9427$) as shown in **Figure 32 C** but not after the GMP drink despite the fact that plasma proline levels were similar after the GMP and the CP drinks.

In the prediabetic group, peaks of proline were also significantly higher after the GMP drink (3.04 ± 0.27 fold) and the CP drink (2.53 ± 0.13 fold) compared to the peaks of the WPI drink (1.46 ± 0.05 fold) and the MD19 drink ($P < 0.0001$, respectively) (**Figure 32 D**). AUC followed those changes as shown in **Figure 32 G**.

Peaks of ornithine were significantly different after ingestion of the WPI drink (1.37 ± 0.08 fold), the CP drink (1.56 ± 0.07 fold) and the GMP drink (1.33 ± 0.07 fold). Also in the prediabetic group the peak of ornithine was elevated after consumption of the CP drink compared to the GMP drink ($P = 0.0226$) despite the fact that the proline peak was higher after the GMP drink compared to the CP drink (**Figure 32 E**) with AUC showing similar changes (**Figure 32 H**) A strong correlation between proline and ornithine was found after the CP drink (**Figure 32 F**) and after the WPI drink ($r = 0.9489$, $P < 0.0001$, $R^2 = 0.9003$).



Proline content in the milk fractions

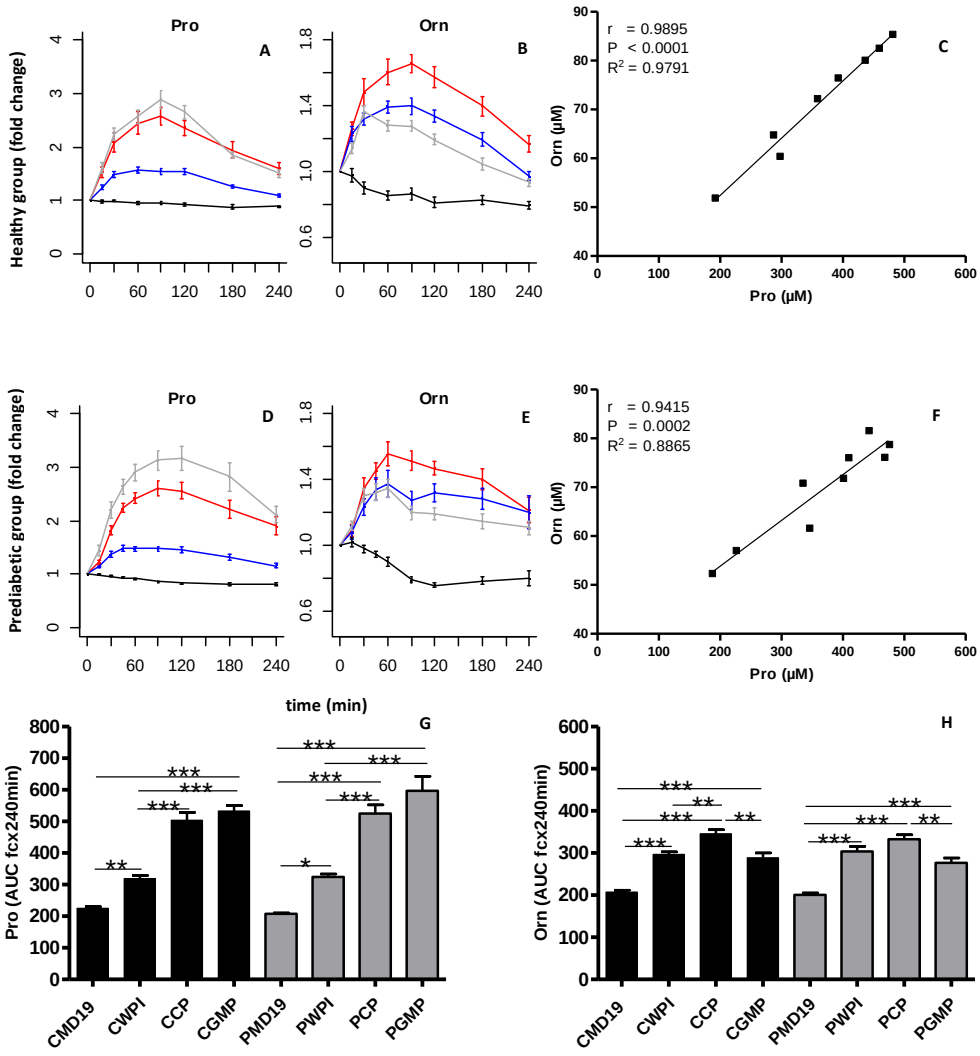


Figure 32: Time courses, AUC and correlations of plasma proline and ornithine after intake of WPI (blue), **CP** (red), **GMP** (grey) **and MD19. (C, F)** On the right side, a strong correlation is illustrated between proline and ornithine after the CP drink in both groups. **Healthy group**: (**A**) Significant differences in proline excursions could be observed between all diets ($P < 0.0010$, for all) except between the CP drink and the GMP drink. (**B**) Ornithine: All diets induced significant differences ($P < 0.0047$, for all) except between the WPI drink and the GMP drink. **Prediabetic group**: (**D**): Significance of proline could not be analyzed with the SAS program. (**E**) Ornithine was significantly different after the WPI drink, CP drink and GMP drink compared to the MD19 drink ($P < 0.0001$ for all). The CP drink induced also a significant difference in ornithine elevations compared to the GMP drink ($P = 0.0117$). Significance was determined by repeated-measures ANOVA treatment effects and treatment x time interactions followed by Tukey Kramer post hoc test. (**E, F**) Pearson's correlations between proline and ornithine after intake of the CP drink in the healthy and prediabetic group. (**G, H**) AUC of proline and ornithine analysed by repeated one way ANOVA and Tukey Kramer post hoc test. Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. (C = control group, P = prediabetic group)

4.7.7 Plasma acylcarnitine levels

The short chain acylcarnitines C3, C4 and C5 derive mainly from BCAA metabolism as shown in **Figure 33**.

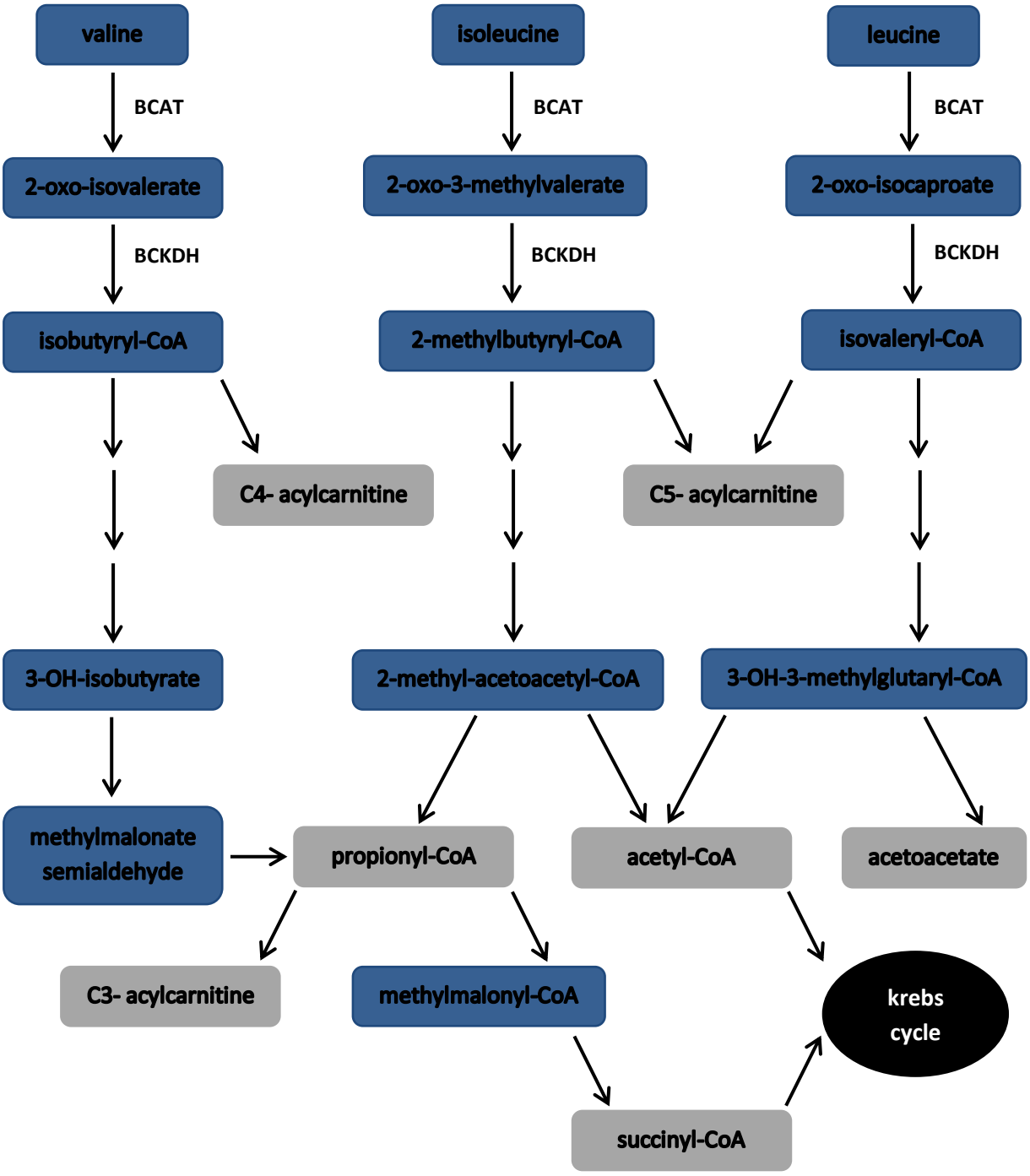


Figure 33: Degradation of BCAAs with production of the corresponding acylcarnitines. Adapted from Newgard et al. 2012 and modified. BCAT, Branched chain amino transferase, BCKDH, Branched chain keto acid dehydrogenase

Plasma acylcarnitine concentrations in healthy volunteers are shown in **Figures 34 A, B, C, D, E**. Free carnitine (C0) levels were slightly elevated after intake of all test drinks without any differences between the drinks (**Figure 34 A**) and plasma concentrations of C2 decreased after all test drinks. GMP induced the most marked decrease with a minimum concentration (0.55 ± 0.02 fold), followed by the CP drink (0.56 ± 0.03 fold), the WPI drink (0.62 ± 0.03 fold) and the MD19 drink

(0.64 ± 0.02 fold) (**Figure 34 B**). Minima were not significantly different between test drinks. Despite the lowest insulin response, the AUC of C2 acylcarnitines was strongest reduced after the GMP drink (-14%) compared to the MD19 drink ($P < 0.01$). The CP drink (-12%) and the WPI drink (-8%) also reduced C2 acylcarnitines compared to the MD19 drink. However, no significant difference could be observed for either (**Figure 34 K**).

In the prediabetic group, plasma acylcarnitine concentrations showed time courses similar to the healthy group as shown in **Figures 34 F, G, H, I, J**. C0 levels also slightly increased after ingestion of all test drinks without any differences between them (**Figure 34 F**).
Acetylcarnitine (C2) levels decreased after all test drinks. Here, the CP drink caused the strongest decrease (0.53 ± 0.02 fold), followed by the GMP drink (0.55 ± 0.02 fold), the WPI drink (0.60 ± 0.03 fold) and the MD19 drink (0.65 ± 0.04 fold). Minimum concentrations of C2 acylcarnitines were significantly different after the CP drink and the GMP drink compared to that observed after the MD19 drink ($P = 0.0072$, $P = 0.0341$, respectively) (**Figure 34 G**). The AUC of C2 acylcarnitines were significantly reduced after the CP drink (-11%) compared to the MD19 drink. The WPI drink (-3%) and the GMP drink (-7%) reduced C2 acylcarnitines only marginal (**Figure 34 K**).

In contrast, propionylcarnitine (C3), butyrylcarnitine (C4) and isovalerylcarnitine (C5) levels increased after all 3 test drinks and decreased slightly after the MD19 drink in the healthy volunteers. Peaks of C3 were highest after the GMP drink (2.21 ± 0.09 fold), followed by the WPI drink (1.68 ± 0.03 fold) and the CP drink (1.50 ± 0.04 fold). Peaks were all significantly different from each other except after the WPI drink compared to the CP drink ($P < 0.0075$, for all) (**Figure 34 C**). Also the AUC of C3 acylcarnitine was greatly increased after the GMP drink (+76%), followed by the WPI drink (+45%) and the CP drink (+30%) compared to the MD19 drink (**Figure 34 L**).
In the prediabetic group, C3 acylcarnitines increased after all test drinks. The strongest peak was also observed after the GMP drink (2.86 ± 0.16 fold), followed by the CP drink (2.36 ± 0.09 fold) and the WPI drink (2.19 ± 0.11 fold). Peaks were significantly different compared to the MD19 test drink ($P < 0.0012$, for all) (**Figure 34 H**). The AUC of C3 acylcarnitines were highest increased after the GMP drink (+72%), followed by the WPI drink (+53%) and the CP drink (+47%) compared to the MD19 drink (**Figure 34 L**).

Plasma levels of C4 peaked after the WPI drink (1.32 ± 0.04 fold), the CP drink (1.39 ± 0.05 fold) and the GMP drink (1.47 ± 0.11 fold) in healthy volunteers. Peaks were significantly different after the WPI drink, CP drink and the GMP drink compared to that observed after the MD19 drink ($P = 0.0135$, $P = 0.0020$, $P < 0.0001$, respectively) (**Figure 34 D**). The WPI drink (+27%), the CP drink (+ 29%) and the GMP drink (+ 32%) caused also a highly significant elevation of the AUC of C4 acylcarnitines in plasma compared to the MD19 drink (**Figure 34 M**).

In the prediabetic group, C4 acylcarnitine levels increased most strongly after the GMP drink (1.45 ± 0.08 fold), followed by the CP drink (1.33 ± 0.06 fold) and the WPI drink (1.32 ± 0.06 fold) at 240 min which was significantly different compared to the MD19 drink (P < 0.0002, for all) (**Figure 34 I**). The WPI drink (+27%), the CP drink (+30%) and the GMP drink (+36%) caused also a highly significant elevation of the AUC of C4 acylcarnitines in plasma compared to the MD19 drink (**Fiugre 34 M**).

In healthy volunteers, levels of C5 acylcarnitine were maximally increased after the WPI drink (1.85 ± 0.48 fold) followed by the CP drink (1.32 ± 0.34) and GMP drink (1.30 ± 0.34 fold). The peak of C5 acylcarnitine was significantly different after the WPI drink compared to that seen after the CP drink, GMP drink and MD19 drink (P < 0.0006, for all) (**Figure 34 E**). The AUC of C5 was most strongly increased after the WPI drink (+ 65%) followed by the CP drink (+ 31%) and the GMP drink (+25%) compared to the MD19 drink (**34 N**).

Correlations between all plasma BCAAs and the corresponding acylcarnitines formed in both groups are provided in **Figure 35**. BCAAs correlated also (not shown) with C0 (r = 0.6841, P = 0.0002, R^2 = 0.4680) and C2 (r = -0.5878, P = 0.0025, R^2 = 0.3455) summarized after all 3 test drinks. Furthermore, a strong correlation was found between C3 and threonine (r = 0.7372, P <0.0001, R^2 = 0.5435).

In the prediabetic group, C5 acylcarnitine was also maximally increased after the WPI drink (1.89 ± 0.13 fold). The peak of C5 acylcarnitine was significantly different after the WPI drink (P < 0.0001), the CP drink (1.56 ± 0.30 fold, P = 0.0004) and the GMP drink (1.41 ± 0.07 fold, P = 0.0124) compared to that after the MD19 drink. The peak of C5 was also significantly different after the WPI drink compared to that after the GMP drink (P = 0.0016) (**Figure 34 J**) with similar changes in AUC values (**Figure 34 N**).

BCAAs correlated also with C0 (r = 0.5751, P = 0.0017, R^2 = 0.3307) and C2 (r = -0.4838, P = 0.0106, R^2 = 0.2341) in the prediabetic group. Here, a strong correlation was also found between C3 and threonine (r = 0.6050, P = 0.0008, R^2 = 0.3660).

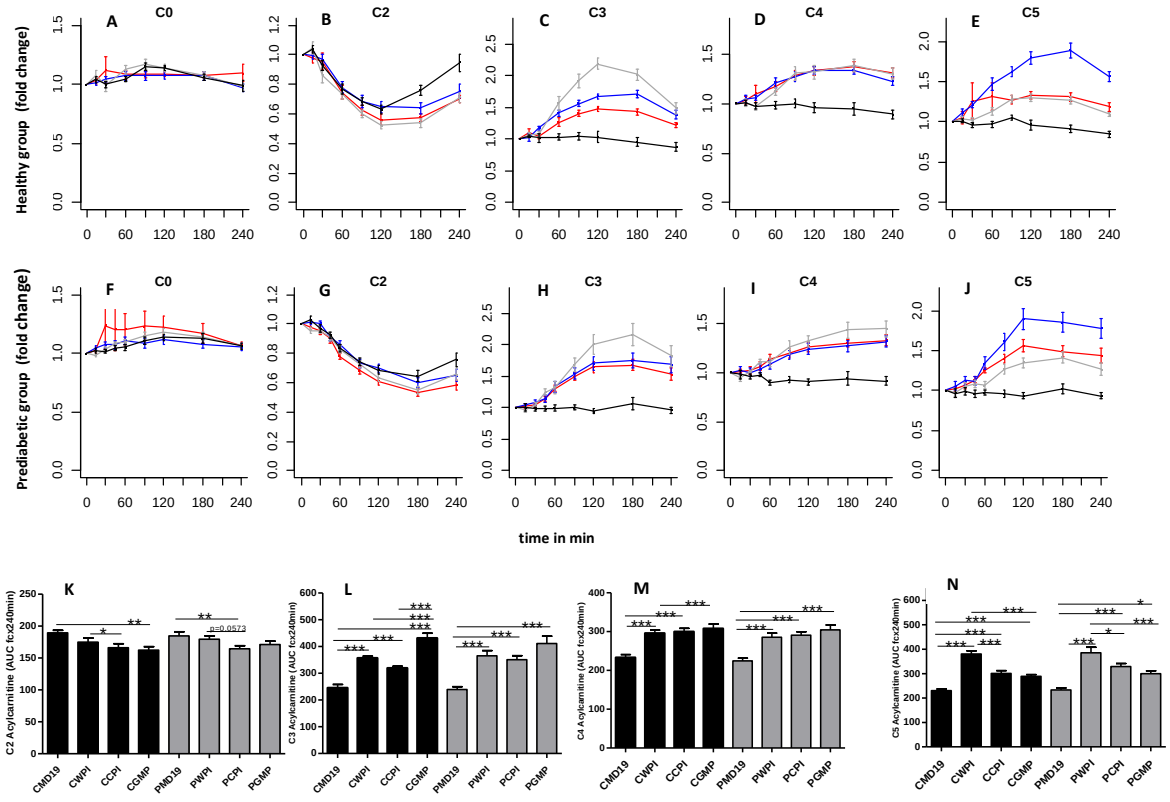


Figure 34: Time course and AUC of plasma acylcarnitines after intake of WPI (blue), CP (red), GMP (grey) and MD19**. Healthy group:** (**A**) C0: not significantly different (**B**) C2: CP drink and GMP drink compared to the MD19 drink ($P < 0.05$, for both), (**C**) C3: WPI drink compared to the CP drink and the MD19 drink ($P = 0.0025$, $P < 0.0001$, respectively). WPI drink compared to the GMP drink ($P = 0.0042$), CP drink compared to the MD19 drink and the GMP drink ($P < 0.0001$, for both), GMP drink compared to MD19 drink ($P < 0.0001$). (**D**) C4: WPI drink, CP drink and GMP drink compared to the MD19 drink ($P < 0.0001$, for all). (**E**) C5: WPI drink compared to the CP drink, GMP drink and the MD19 drink ($P = 0.0033$, $P < 0.0001$, $P < 0.0001$, respectively), GMP drink compared to the MD19 drink ($P < 0.0001$). **Prediabetic group:** (**F**) C0: not significantly different (**G**) **C2:** CP drink compared to the MD19 drink ($P < 0.05$). (**H**) C3: WPI drink, CP drink, GMP drink compared to the MD19 drink ($P < 0.0001$, for all). (**I**) C4: WPI drink, CP drink, GMP drink compared to the MD19 drink ($P = 0.0003$, $P < 0.0001$, $P = 0.0003$, respectively). (**J**) C5: WPI drink compared to the GMP drink and the MD19 drink ($P < 0.0001$, $P = 0.0005$, respectively), CP drink and GMP drink compared to the MD19 drink ($P < 0.0001$, $P = 0.0099$, respectively). Significance was determined by repeated-measures ANOVA treatment effects followed by Tukey Kramer post hoc test. (**K, L, M, N**): AUC of C2, C3, C4 and C5 from both groups. AUC was analysed by one way ANOVA and Tukey Kramer post hoc test. Significance: *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. (C = control group, P = prediabetic group).

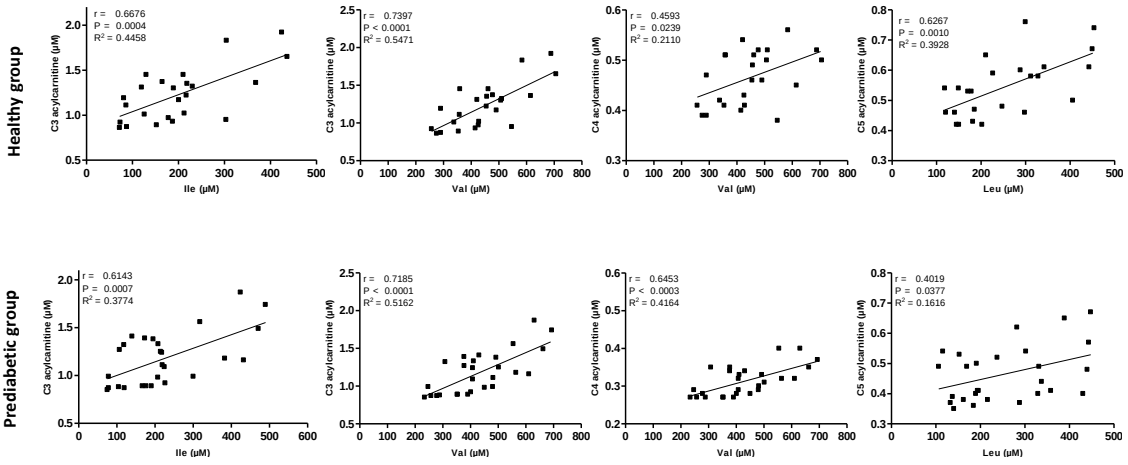


Figure 35: Pearson's correlations between plasma BCAAs after intake of all milk fraction drinks from the healthy and prediabetic group and the corresponding plasma acylcarnitines

4.7.8 Cross-correlation analysis of plasma responses

Cross-correlation matrices of all amino acids and acylcarnitines are shown in **Figure 36**. The CP and GMP drinks generated more positive associations compared to the WPI drink in healthy and prediabetic volunteers. The correlation matrix after the GMP drink demonstrates a completely different pattern based on the lack of aromatic amino acids and a lack of degradation products thereof.

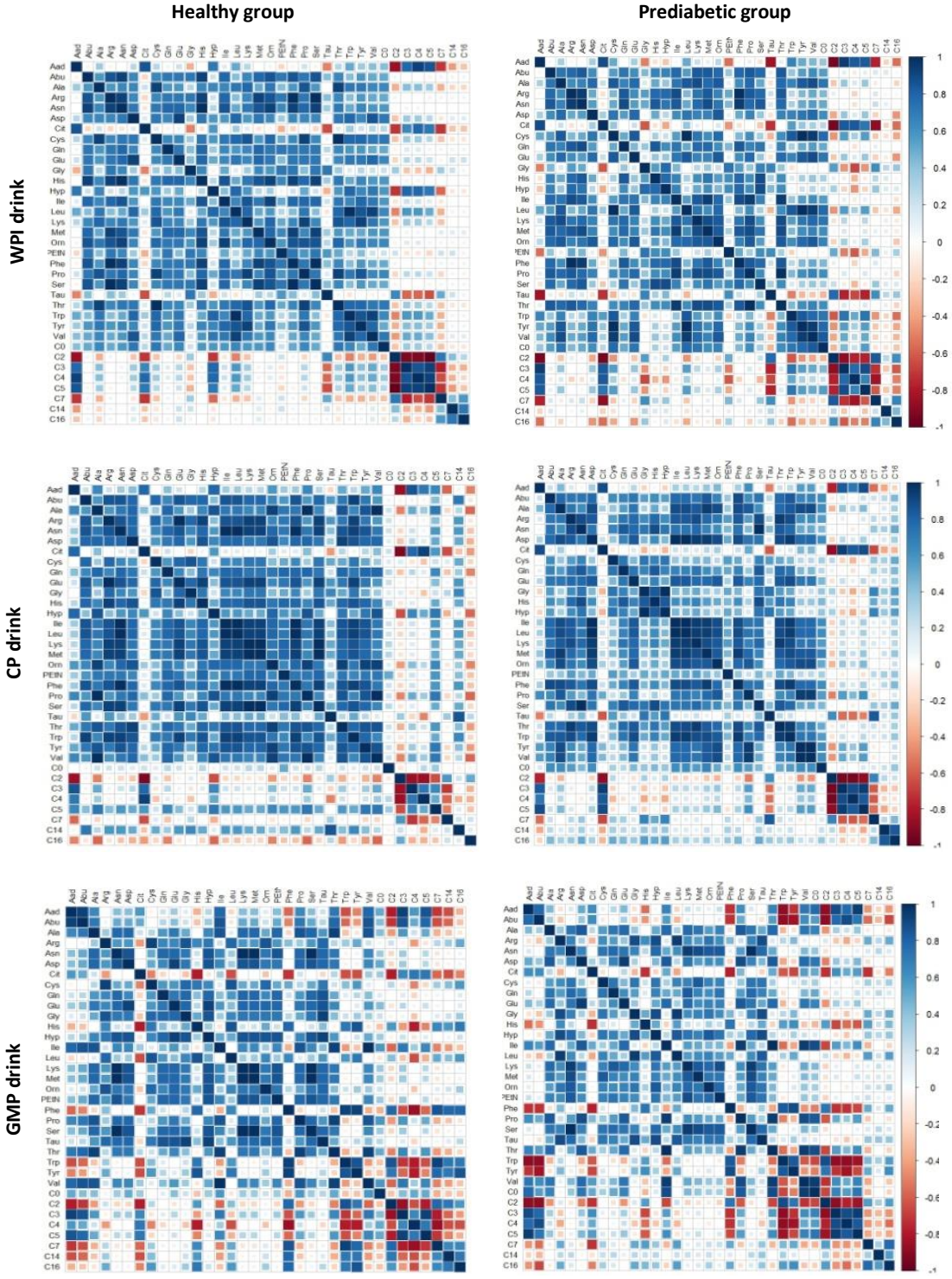


Figure 36: Kendall's correlation matrices of plasma amino acids and acylcarnitines after ingestion of the different milk fraction drinks in healthy and prediabetic volunteers.

5 Discussion

Protein ingestion enhances glucose-induced plasma insulin responses in healthy volunteers (Krezowski et al. 1986) and in patients with non-insulin dependent diabetes (Gannon et al. 1988). The insulinotropic effect is thought to originate from increased insulin output and enhanced uptake of glucose in striated muscles and adipose tissue mediated by amino acids (Watson et al. 2001, Doi et al. 2003). In contrast, protein intake alone does not alter blood glucose levels despite the fact that it slightly elevates plasma insulin levels (Nuttall et al. 1984, Gannon et al. 1992). Amongst different dietary proteins, whey proteins were shown to be the most potent in enhancing the insulin response and lowering blood glucose and this is attributed mainly to the high BCAA content and excellent digestibility (Pal et al. 2010, Morifuji et al. 2010).

Casein, that has similar amounts of BCAAs, differs in its physicochemical properties and precipitates in the acidic environment of the stomach. Furthermore, proteolytic cleavage of the κ-casein fraction releases the glycomacropeptide causing a more hydrophobic environment promoting the aggregation of casein micelles and the formation of a gel (Lebensmittelchemische Gesellschaft, 1991). A delayed gastric emptying after administration of casein has been shown in rodents (Miranda et al. 1983, Daniel et al. 1990) but also in human studies, Boirie et al. classified in 1997 whey protein as a "*fast*" and casein as a "*slow*" protein based on different kinetics of leucine appearance in plasma (Boirie et al. 1997, Frühbeck 1998). Glycomacropeptide (GMP), containing 64 amino acids, is thought to be more resistant to proteolytic cleavage in the gastrointestinal tract based on its glycosylation and high amount of proline residues and its feature to inhibit gastrin output and thus gastric acid secretion (Chabance et al 1998, Stan et al. 1982, 1983). Extraordinary for GMP is the lack of the aromatic amino acids phenylalanine, tyrosine and tryptophan and simultaneously the very low abundance of arginine, cysteine and histidine (Stan et al. 1982, 1983). Although interesting as a peptide, the fate of GMP and its effect on postprandial metabolism in humans has not been studied before.

The present study therefore aimed to carefully analyse the postprandial metabolic responses to whey protein, casein and GMP in healthy and prediabetic subjects in a comparative approach with each individual serving as its own control.

5.1 Effects of the test drinks on glucose homeostasis and hormone secretion

5.1.1 Relations between postprandial insulin increase and glucose disposal

All test drinks reduced blood glucose concentrations compared to the MD19 drink and this effect was much more pronounced in prediabetics. However, no difference in the lowering of blood glucose levels was observed between the different protein sources. Similar findings with a lack of a difference between the protein species have been reported before (Acheson et al. 2011, Hall et al. 2003, Tessari et al. 2007, Gunnerud et al. 2012). In this respect, our study also failed to confirm the concept that whey protein is a "*fast*" and casein as a "*slow*" digestible protein (Boirie et al. 1997, Beaufrère et al. 2000) because we could not detect any differences in transit time or digestion and absorption as judged by identical plasma appearance rates of the amino acids.

That whey and casein strongly elevated insulin levels matches well with previous findings (Hall et al 2003, Tessari et al. 2007, Acheson et al. 2011). However, despite significantly elevated insulin concentrations, appropiate glucose excursions were not detected. Such a a discrepancy between the increase in insulin concentrations and glucose changes especially after intake of whey protein have been described before (Liljeberg et al. 2001, Hoppe et al. 2009, Post-Skagegard et al. 2006). Remarkably, the GMP drink induced the strongest decrease in blood glucose despite a rather modest insulin response and this was observed in both the healthy and prediabetic volunteers. Effects of GMP on glucose homeostasis have so far only been studied in mice and demonstrated a decrease in plasma insulin with increasing intake of GMP (Royle et al. 2008). Since GMP does not contain phenylalanine and only small amounts of arginine, with both amino acids known to elicit insulin secretion (Floyd et al. 1966, Blachier et al. 1989, Landgraf et al. 1974), the lower insulin output may be raleted to that. Other findings suggested that BCAAs and phenylalanine may act synergistically with glucose to potentiate insulin secretion (Power et al. 2009, Calbet et al. 2002). However, plasma C-peptide concentrations argue against a distinct role of these amino acids in insulin output since they did not differ between casein and GMP in either volunteer group. It thus seems rather an effect on glucose removal from blood that may be affected by the unusual amino acid profile in plasma after GMP ingestion. For example, plasma isoleucine levels after GMP are twice as high than after whey or casein ingestion and recently, the dipeptide L-leucyl-L-isoleucine and free isoleucine were shown to increase GLUT4 translocation independent of insulin (Morato et al. 2013). Oral administration of isoleucine has been demonstrated to provoke a hypoglycemic effect via increased muscle glucose uptake, glucose oxidation and diminished hepatic gluconeogenesis in rats and also in humans (Doi et al. 2007, Nutall et al. 2008). The reduction in blood glucose was more pronounced in mice with diabetes than in healthy mice (Doi et al. 2005), which is consistent with our findings in humans. Alanine is the preferred amino acid for gluconeogenesis (Felig et al. 1973). GMP possesses a similar amount of

alanine as whey protein. In comparison to the WPI drink, this amino acid is significantly elevated in plasma of prediabetic subjects after the GMP drink, which could be linked to a lower rate of gluconeogenesis. Proline as well, which has a high abundance in GMP, has also been described to lower blood glucose without an increase in insulin response (Nutall et al. 2004).
The peptide hormone bradykinin has been described to increase insulin sensitivity, affect insulin release and clearance and this kinin is inactivated by angiotensin-converting enzyme (ACE) (Damas et al. 1999). Several peptides are known to inhibit ACE, such as Ile-Pro-Ala (Abubakar et al. 1998) and GMP provides the highest amount of these three amino acids. Whether this axis has any significance when GMP is ingested deserves further studies. The GMP drink also caused the strongest decrease in NEFAs and β-HB which supports the notion that GMP can increase insulin activity with NEFAs and β-HB known to cause a disturbance of glucose phosphorylation (Williamson & Krebs 1961, Felts et al. 1964) and glucose accumulation. Taken together, our results and those from literature argue for a beneficial role of GMP by enhancing insulin sensitivity and promoting glucose utilization.

In the prediabetic group, the elevation of venous blood glucose after intake of the test drinks was more prolonged and clearance was more delayed when compared to healthy subjects. Insulin is secreted in 2 phases, whereupon in the first phase small amounts of insulin are released over a 10 minute period. First phase of insulin secretion and the pulsatile rate are known to be altered in people with impaired glucose tolerance and type 2 diabetes, which induce a delayed and more prolonged plateau in the glucose and insulin response and an augmented second-phase insulin action (Keefe et al. 2007, Polonsky et al. 1996, Bagger et al. 2011). This is in accordance with our findings in the prediabetic volunteers. The AUC of venous blood glucose levels were higher in prediabetics after MD19 drink but not when proteins were coadminstered. This also argues for a role of postprandial amino acids in modulating glucose uptake from blood.

The AUC of C-peptide was similar between the two volunteer groups in response to MD19 drink but increased after whey and casein intake in the prediabetic group. This is consistent with the findings that protein intake per se enhances glucose sensitivity of the endocrine pancreas (Linn et al. 2000). Plasma glucose after the MD19 drink fell down below basal levels later than after the protein drinks in both groups supporting as well that protein intake and plasma amino acid changes can increase glucose removal and utilization independent of the prevailing insulin level as shown by others as well (Wang et al. 2012, Nuttal et al. 2008).

The hepatic extraction of C-peptide is negligible and therefore plasma C-peptide concentration is a valid indicator of insulin secretion (Stoll et al 1970, Faber 1978). Fifty percent of portal insulin is eliminated during first pass transit and prolonged elevation in portal insulin leads to a reduced clearance due to receptor downregulation (Duckworth et al. 1998). In the present study, both volunteer groups displayed a higher insulin/C-peptide ratio after the WPI drink when compared for example to

GMP. Such higher insulin/C-peptide ratios after whey protein intake or leucine administration have been demonstrated previously (Pidhainy & Wolever 2010, Gibby et al. 1983). Leucine has been reported to suppress protein degradation in liver and muscle, and for casein an inhibition of insulinase was observed which could be responsible for the diminished degradation rate of insulin (Greiwe et al. 2001, Lundholm et al. 1981, Sacks et al. 1977). Furthermore, administration of leucine is known to promote insulin resistance (Tremblay et al. 2001) which provokes hyperinsulinemia and a reduced insulin clearance rate as shown by Bonora et al. (Bonora et al. 1983). The higher insulin response could be attributed either to a disturbed insulin degradation induced by particular amino acids or by a specific leucine-induced insulin resistance. Our findings in summary suggest that the higher insulin levels in blood observed after whey and casein intake are mainly the effect of a reduced clearance rate of insulin and are not predominantly an effect on insulin secretion.

5.1.2 Effects of the test drinks on plasma incretin hormones

Glucose, fatty acids and amino acids promote insulin secretion by directly stimulating pancreatic β cells to release insulin. However, they as well can do this in an indirect manner mediated by the incretin hormones GIP and GLP-1 which are secreted by enteroendocrine cells in response to nutrient intake (Fehmann et al. 1995). However, the potential of dietary proteins or protein hydrolysates thereof to stimulate GIP and GLP-1 output is discussed controversially. Proteins are regarded as the weakest secretagogues for incretin release (Reimann et al. 2010) although an increase of GIP was reported in humans after ingestion of whey protein and casein. The authors suggest that peptides or proteins are necessary for the stimulation of GIP because its secretion was significantly lower after administration of free amino acids when added to a mixed meal (Tessari et al. 2007). In our study, total plasma GIP levels were significantly lower after casein and GMP intake compared to whey. The lack of aromatic amino acids in GMP does not seem to play a crucial role here because incretin responses were similar to those observed after casein although aromatic amino acids were shown to increase GIP secretion in rats (Mace et al. 2012). Correlations between GIP concentrations and most of the plasma amino acids in the healthy group were similar for whey and casein except for proline, threonine, tyrosine, ornithine and valine. These amino acids only showed correlations with GIP after intake of the WPI and total plasma GIP showed a biphasic pattern after the WPI and GMP drinks in the healthy but not in the prediabetic group. The GMP drink did not induce a strong GIP response neither in healthy nor in prediabetic subjects. This may be related to reported effects of GMP to inhibit gastrin output and gastric acid secretion (Stan et al. 1982), both shown to be essential for the secretion of GIP (Wolfe et al. 2000).

Regarding plasma GLP-1, it exhibited a biphasic pattern after ingestion of the WPI and GMP drinks in healthy volunteers. The first GLP-1 peak appears after 30 min and is suggested to be mediated by a neuronal stimulus since GLP-1-secreting L-cells are predominantly found in the distal part of the

intestine. The vagus nerve has been shown to be a crucial mediator of nutrient stimulated GLP-1 secretion (Rocca et al. 1999, Roberge et al. 1996). We here observed a strong correlation between plasma GIP and GLP-1 concentrations after intake of casein and whey suggesting that GIP is involved in the proximal-distal loop mediating stimulus secretion coupling (Rocca et al. 1999). Some human studies observed only higher levels of GIP but not GLP-1 after administration of whey protein when compared to a reference meal containing only carbohydrates (Frid et al. 2005, Nilsson et al. 2004), whereas others report a stimulation of both incretins (Tessari et al. 2007, Hall et al. 2003) as shown here.

The underlying mechanism causing incretin hormones to increase after whey protein ingestion may be related to findings in rodent models. Whey administration in mice revealed a significant reduction of DPPIV activity in the proximal intestine, where GIP-secreting cells are located, whereas no change was monitored in the distal gut or in plasma. Although total GIP secretion was unchanged and total GLP-1 release was increased, elevated levels of active GLP-1 and active GIP were found in the mice suggesting whey to alter incretin kinetics (Gunnarsson et al. 2006) with DPPIV activity shown to be the determinant of active GIP (Carr et al. 2010). Bioactive peptides such as Ile-Pro-Ala (IPA), released from β-lactoglobulin after hydrolysis were shown to inhibit dipeptidylpeptidase IV *in vitro* (Tulipano et al. 2011) so were tripeptides such as Leu-Pro-Leu released by casein digestion shown to inhibit DPPIV (Power et al. 2014). Whether those peptides can *in vivo* affect DPPIV and in turn active incretin levels needs to be established. The role of specific peptides and amino acids in eliciting hormone release from endocrine cells is also not defined. Perfusion of the amino acids phenylalanine, tryptophan, asparagine, arginine and glutamine into small intestine of rats elevated total plasma GIP concentration (Mace et al. 2012) and peptones as an undefined mixture of peptides were also shown to increase GIP output in dogs and rats (Wolfe et al. 1982, Wolfe et al. 2000) although the mechamisms are not yet clear. However, our findings that the milk protein fractions promote an increase of incretin hormones in plasma either by direct stimulation of secretion in the gut or by inhibition of DPPIV which rapidly inactivates GIP and GLP-1 asks for more research on those effects.

5.1.3 Effects of the test drinks on glucagon levels

Plasma glucagon levels were found elevated in healthy and prediabetic subjects upon ingestion of the test drinks but without any differences between them. MD19 alone caused a decrease in plasma glucagon as previously described (Claessens et al. 2009, Acheson et al. 2011). This suggests that a high blood glucose concentration is primarily suppressing glucagon output (Walker et al. 2011) to which paracrine signals may contribute. Despite a strong rise of insulin after MD19 intake a slight increase in glucagon levels became visible in the prediabetic group. This matches with findings that a rise in glucose can elicit glucagon secretion from pancreatic islets from donors with type 2 diabetes (Walker et al. 2011) and this may originate from a down-regulation of insulin receptors on intra-islet alpha cells by chronic exposure to high concentrations of insulin (Franklin et al. 2005, Kisanuki et al.

1995, Pipeleers et al. 1985) suppressing inhibitory insulin effects on alpha cells and thus promoting glucagon secretion.

Protein ingestion potentiates glucagon secretion in the presence of carbohydrates and this is thought to prevent a hypoglycemic state by affecting mainly hepatic glucose production (Unger et al. 1969). Thus, dietary protein intake increases both, insulin and glucagon secretion, whereas glucose alone inhibits glucagon secretion (Ahmed M. et al. 1980, Kabadi et al. 1991, Calbet and MacLean 2002). Protein has been shown to be a stronger secretagogue for glucagon secretion than glucose in repressing it (Westphal et al. 1990). Although some amino acids induce gluconeogenesis, several studies revealed no or only a slight change in blood glucose levels after protein ingestion (Gannon et al. 1992, Nuttall et al. 1991, Krebs et al. 2003). Intringuingly, a concomitant activation of hepatic gluconeogenesis and glycogenesis was demonstrated in rats after a high protein diet (Azzout-Marniche et al. 2007), which would explain the missing increase in blood glucose because glucose is sequnetered to glycogen. Particular amino acids like alanine, glutamine and proline have been shown to stimulate glycogen synthesis in hepatocytes of rats, and this is partly mediated via an increase in cell volume caused by amino acid uptake. Baquet et al. reported that osmotic cell swelling itself induces glycogen synthesis (Baquet et al. 1990) and amino acids promote the gluconeogenic flux of glucose-6-phosphate from glucose formation to UDP-glucose and glycogen (Katz et al. 1984). Intriguingly, glycogen synthesis in hepatocytes is more related to gluconeogenic C-3 units, whereas glucose is more the activator for the generation of glycogen in the liver known as the glucose paradox (Katz et al. 1984). In contrast to the liver, a higher formation of glycogen in muscle was observed after a high protein intake and simultaneous carbohydrate supplementation compared to normal or low carbohydrate ingestion in man (Ivy et al. 2002). We also observed in the present study the strong compensation mechanism of glucagon output to safeguard glucose homeostasis. The release of glucagon depends obviously on the protein/carbohydrate ratio in the meal and is suppressed when this ratio is low (Day et al. 1978). In our study the ratio was 1:1 leading to the highest insulin/glucagon ratio after MD19 followed by the casein, whey protein and GMP drinks. MD19 thus resulted in the highest insulin/glucagon ratio while blood glucose was lowest after GMP with the lowest insulin/glucagon ratio.

Whereas essential amino acids in a dietary protein appear to promote insulin secretion, non-essential amino acids and arginine elicit more glucagon secretion (McCarty et al. 1999) and alanine, arginine and glutamine were shown to be the most potent (Muller et al. 1971, Pipeleers et al. 1985) but also AAAs, tyrosine and BCAAs are effective (Rocha et al. 1972, Claessens et al. 2009). In our human volunteers, we observed strong correlations between glucagon and most of the amino acids in plasma. Despite the fact that aromatic amino acids are absent in GMP, this glycopeptide elicited the strongest glucagon response suggesting that aromatic amino acids are not relevant for glucagon output *in vivo*.

Glucagon secretion is also regulated by the incretin hormones GIP and GLP-1 (De Heer et al. 2008). However, the different incretin responses in our volunteers did not relate to glucagon secretion and we therefore assume that certain amino acids in interplay with glucose are predominantly responsible for glucagon secretion.

5.1.2 Effects of the test drinks on the feeling of hunger

Healthy volunteers showed the lowest feeling of hunger after consumption of the WPI followed by the GMP and CP drinks. Satiety effects of whey protein have been attributed to increased incretin hormones, CCK, thermogenesis and an increase of specific plasma amino acids (Bowen et al. 2006, Hall et al. 2003, Veldhorst et al. 2009). Furthermore, opioid receptors activated by peptides released from poteins during digestion have also been suggested to contribute to the satiety effects (Pupovac et al. 2002, Froetschel et al. 2001). Hall et al. demonstrated a stronger satiety and lower ad libitum energy intake after a whey protein preload, compared to an equivalent casein preload which was accompanied by significant increases of plasma amino acids, GLP-1 and CCK levels (Hall et al. 2003). Satiety effects of whey have also been attributed to GMP (Burton-Freeman et al. 2008) but others failed to demonstrate such an effect (Poppitt et al. 2013, Chungchunlam et al. 2014).

Insulin is a postprandial satiety hormone. At low concentrations it elicits satiety but it induces a feeling of hunger at high plasma concentrations and the insulin levels reached in our study would argue for a suppressive action on the hunger feeling (Anika et al. 1980, Vanderweele 1994). GMP produced some interesting findings with a strong reduction in ghrelin levels and the feeling of hunger. Geary and colleagues proposed that glucagon, next to CCK and GLP-1 is one of the most potent mediators of satiety (Geary et al. 1990) and it reached highest levels after GMP intake in healthy subjects. Since protein ingestion increases glucagon levels more than equicaloric amounts of carbohydrates which tend to diminish glucagon secretion, the satiety effect of proteins was linked to glucagon secretion and the initiation of gluconeogensis (Belza et al. 2013, Booth et al. 1970) by glucagon. Moreover, a depression of ghrelin levels by subcutaneously injected glucagon has been demonstrated (Soule et al. 2005). Plasma glucagon levels in the present studies showed a negative correlation with ghrelin levels after the WPI and CP drinks suggesting a negative feedback loop between glucagon and ghrelin output in control of satiety.

For decades one of the most prominent hypotheses in satiety control was the serotonin paradigm proposed by Fernstrom and Wurtman. According to this hypothesis (Fernstrom & Wurtman 1971, 1972), availability of tryptophan (Trp) to brain cells for serotonin synthesis would control satiety and determine the preference for either carbohydrate- or protein-rich foods in the next serving. Trp availability to brain is affected by various determinants. It competes with other long neutral amino acids (LNAAs) such as leucine, valine, isoleucine, phenylalanine and tyrosine for uptake into cells via the amino acid transporter LAT1 (Oldendorf et al. 1976). LNAAs uptake via LAT1 is insulin

dependent and in the postprandial state with high insulin levels, LNAAs influx is increased but Trp uptake not, as Trp it is bound to albumin. This in turn enables larger amounts of Trp to pass through the blood-brain barrier leading to increased formation of serotonin in the brain and suppression of food intake (Teff et al. 1988, Halford et al. 2007) via an activation of 5-HT_{2C}Rs (5-hydroxytryptamine$_{2c}$ receptors) in POMC neurons, (Pissios et al. 2007). In context of the Wurtman hypothesis the concentration ratio of Trp over LNAAs has been used as a predictive marker for enhanced serotonin synthesis.

Based on the use of GMP that provides no Trp to systemic circulation whereas whey and casein deliver Trp we had a unique setting to test whether the hypothesis of Fernstrom and Wurtman in providing satiety by the protein-containing test drinks which has an association with the different plasma amino acid levels. We therefore calculated the ratio between Trp and the LNAAs with the lowest Trp/LNAA ratio as expected after GMP and a Trp/LNAA twice as high after whey than after casein intake by a two-fold higher amount of Trp in whey protein than in casein. The highest Trp/LNAA ratio was observed after the MD19 drink despite the fact that Trp levels did not increase. However, it needs to be mentioned that the Trp/LNAA ratio has to increase more than 50% to induce a powerful elevation of Trp in the brain (Ashley et al. 1985, Teff et al. 1989). Although the Trp/LNAA ratio after the GMP drink decreased by around 70%, the feeling of hunger was not different than after ingestion of the other proteins. This argues against a significant effect of the Trp/LNAA ratio or the Wurtman hypothesis in satiety control and leaves the suggestion that elevated glucagon levels in the presence of proteins in a test diet may mediate most of the well-known satiety effects of proteins.

5.3 Gastrointestinal transit time effects

5.3.1 Impact of the test drinks on gastric emptying

Increases in blood glucose concentrations following carbohydrate intake depend substantially on the rate at which carbohydrates leave the stomach and the variance in postprandial plasma glucose is attributable by 34% to the gastric emptying rate (Horowitz et al. 1993). As described before, whey protein is considered a fast and casein a slowly digestible protein (Boirie et al. 1997, Frühbeck 1998). This classification was initially based on the observation that leucine appeared in plasma of humans with a sharp postprandial rise after whey intake, whereas a slower but prolonged plateau in leucine levels was found after casein intake. Differences in gastric emptying between whey and casein were also observed in two other more recent human studies (Hall et al. 2003, Acheson et al. 2011).

In the present study neither the appearance of hydroxyproline in plasma in healthy volunteers nor of acetaminophen in prediabetic volunteers showed any marked differences in appearance as a function of protein type ingested. Furthermore, casein and whey revealed no differences on the postprandial insulin response and glycemia confirming similar previous findings (Acheson et al. 2011, Boirie et al.

1997). The time-dependent changes in plasma amino acid levels did also not reveal any differeces between whey and casein – except for differences caused by the different amounts present in the test proteins – suggesting again that gastrointestinal handling of the applied proteins including gastric emptying and transit is not different. This finding, of course, contradicts the expected differences and those of previous human studies. As a possible explanation may serve the behavior of the different forms of caseins which have different precipitation characteristics. We used a Na-caseinate whereas in most other studies casein in micellar form was applied. While micellar casein is usually obtained by micro- and ultrafiltration, calcium or sodium caseinates are obtained by acid precipitation leading to a denaturation of the protein. This increases its solubility which in turn may render digestion characteristics more similar to whey protein (Post et al. 2012, Phillips 2011). Barbé et al. could clearly demonstrate that rennet gels differ in the coagulation behavior and amino acid availability compared to gels obtained by acidification (Barbé et al. 2014). The lack of a difference was also reported by Calbet et al. (2004), Bowen et al. (2006) and Woodley et al. (2008) which used in their studies also caseinates. In *our vitro* studies we observed modest differences in the buffering capacity of the proteins but otherwise protein behaviours did not show major differences. Besides the protein content in the test diets other factors such as energy density, temperature or osmolarity may also determine the rate of gastric emptying (Calbet et al. 1997, Calbet 2004, Tessari et al. 2007). When determining the osmolality of the test drinks, that of casein was higher by the sodium in the casein when compared to whey. However, Calbet et al. demonstrated that caloric density affects gastric emptying more than osmolality (Calbet et al. 1997) and since energy density was identical in our study, this may as well explain a lack of difference. The GMP drink possessed a higher osmolality but similar caloric density and pH as the WPI and CP drinks. However, GMP left the stomach more rapidly and this may relate to its reported effects on gastric acid secretion (Stan et al. 1982) with a less acidic pH accelerating gastric emptying (Lin et al. 1990).

5.3.2 Alterations of oro-cecal transit time evoked by different test drinks

We used the lactulose breath test to assess oro-cecal transit time (OCTT) which correlates well with transit time of a complex meal assessed by gastroenterocolonic scintigraphy or polyethylene glycol (Read et al. 1985, Bond et al. 1974). However, it has to be taken into account that lactulose itself accelerates transit time by retaining for osmotic reasons fluid in the intestine (Miller et al. 1997, Read et al. 1982). The assessment of the oro-cecal transit time is influenced by many factors. To take some of the confounders into account, OCTT was assessed via the inflection point of the hydrogen concentration curve in breath which shows sigmoidal behaviour. OCTT was fastest for GMP followed by MD19, the casein-containing drink and the whey drink. Since whey ingestion caused the largest GLP-1 increase and GLP-1 is known to inhibit gastric emptying and motility, and it is also known to be involved in the ileal break (Nauck et al. 1993, Nauck et al. 1997, Schirra et al. 2006) a slow transit time may be GLP-1 mediated. A prolonged oro-cecal transit time was observed after all test drinks in

the prediabetic volunteers and this is known to result from a hyperglycemia (Rayner et al. 2001, Russo et al. 1996, Pilotto et al. 1995).

5.4 Postprandial changes in plasma amino acids and metabolites

5.4.1 Branched chain amino acids and their putative effects

As outlined in the introduction, there is a controversial debate on the role of leucine as a ligand of mTor signaling that activates $p70^{S6k}$ kinase and causes serine/threonine phosphorylation of IRS-1 causing insulin resistance (Tremblay 2001, Torrazza et al. 2010). Both, casein and whey ingestion caused very large increases in plasma leucine levels and those were associated with very high systemic insulin levels. The question now arises if this increase in leucine contributes to the development of insulin resistance. Hyperinsulinemia has been observed in individuals after administration of leucine especially in combination with increased glucose intake (Kalogeropoulou et al. 2008, Greiwe et al. 2001, DiGeorge et al. 1960) and amino acids were shown to reduce the sensitivity of insulin to control hepatic glucose output (Flakoll et al. 1992). Most interestingly, infusion of leucine in humans was demonstrated to induce phosphorylation of $p70^{S6k}$ kinase with increased ribosome genesis and promotion of protein translation and a simultaneous infusion of insulin and leucine increased the phosphorylation state of $p70^{S6k}$ (Greiwe et al. 2001). Although insulin causes phosphorylation of protein kinase B (PKB) at $serine^{473}$, the simultaneous presence of high leucine did not change PKB phosphorylation state (Greiwe et al. 2001). Despite higher plasma leucine levels after ingestion of the WPI drink in our study, changes in blood glucose levels were very similar between casein and whey.

The results regarding GMP effects are again interesting. It produced a comparable reduction of blood glucose levels as whey and casein and produced a six fold increase of plasma isoleucine levels. Intriguingly, Doi et al. demonstrated that isoleucine but not leucine increased glucose uptake into skeletal muscle by 73% independent of insulin (Doi et al. 2005) and a decrease of blood glucose levels in the absence of an insulin response after isoleucine intake in humans was also reported (Nuttall et al. 2008). Isoleucine was shown to reduce AMPKα2 activity (Krause et al. 2002). AMPK inactivates PKB and mTOR signaling and reduces $p70^{S6k}$ phosphorylation and protein synthesis for example in skeletal muscle (Bolster et al. 2002, Asish et al. 2010). This raises the question why isoleucine decrease blood glucose despite a reduced AMPK activity that promotes mTOR activation and phosphorylation of $p70^{S6k}$ that is known to promote insulin resistance. Doi et al. postulated that PI3K and PKC but not mTOR mediate the enhanced glucose uptake induced by isoleucine (Doi et al. 2003, Doi et al. 2005). That leucine as well can affect glucose uptake via PI3-kinase and PKC and thus independent of the pathways that cause translocation of GLUT4 by insulin has also been shown (Nishitani et al. 2002).

5.4.2 Plasma α-aminobutyrate as a marker of threonine degradation

After GMP ingestion, we observed in both volunteer groups a marked increase in plasma α-aminobutyrate levels. This confirms previous findings of Veldhorst et al. showing increased α-aminobutyrate levels after administration of whey with GMP as compared to a breakfast with whey but without GMP (Veldhorst et al. 2009). Alpha-amino-n-butyric acid is a non-proteinogenic amino acid and is derived from the metabolism of methionine, threonine, serine or glycine. Elevated concentrations of α-aminobutyrate serve as nonspecific markers of liver dysfunction, malnutrition, alcoholic liver disease or increased protein catabolism (Chiarla et al. 2011). Since GMP also elicited a strong increase of threonine in plasma and this increase correlates well with the alpha-aminobutyrate concentration, we may conclude that it is a marker of threonine degradation. Threonine can be metabolized via threonine dehydrogenase forming 2-amino-3-ketobutyric acid and finally acetyl CoA and glycine but this pathway seems of minor importance (Darling et al. 2000). Threonine can also be catabolized in the cytosol via threonine dehydratase to 2-ketobutyric acid which undergoes transamination to 2-aminobutyric acid by alanine aminotransferase. However, it can also enter the degradation pathway of BCAAs in which it is further metabolized to propionyl coenzyme A by the branched chain ketoacid dehydrogenase or pyruvate dehydrogenase (Darling et al. 1999). Strong correlations were observed between isoleucine and Abu, valine and Abu, valine and isoleucine, valine and leucine after whey intake suggesting a shared pathway of BCAA and ketobutyrate metabolism.

5.4.3 Plasma aminoadipic acid as a marker of lysine degradation

Aminoadipic acid is formed mainly from lysine degradation and increases in plasma when protein degradation is increased (Requena et al. 2001). However, not very much is known about the metabolism of aminoadipic acid. Plasma levels increased 7-fold after intake of whey for example in the prediabetic group. In mice aminoadipic acid was shown to lower blood glucose by enhancing insulin secretion. A strong correlation with kynurenic acid levels was observed (Wang et al. 2013) and interestingly, α-aminoadipic acid was demonstrated to lower extracellular kynurenic acid levels (Wu et al. 1995). Kynurenic acid can block glutamate receptors which are involved in insulin secretion and quinaldic acid derived from kynurenic acid blocks pro-insulin formation and insulin release (Stone et al. 2002). This suggests that α-aminoadipic acid via competition with kynurenic acid may affect insulin secretion. This completion may well explain the significant correlation between plasma aminoadipic acid and tryptophan - which is a precursor of kynurenic acid - after intake of the whey drink in in the prediabetic group.

5.4.4 Plasma free fatty acids and β-hydroxybutyrate levels

Elevated insulin levels cause inhibition of lipolysis associated with a decrease in concentrations of non esterified fatty acids (NEFAs) and thus all test diets that all increased insulin output also suppressed NEFA levels. A remarkable effect however was the strong decline of NEFA levels despite a low insulin response and highest glucagon levels after the GMP drink. Animal studies revealed reduced lipid concentrations in plasma and liver after administration of GMP in rats (Xu et al. 2013, Kim et al. 2005). Remarkably, we could observe strong negative correlations between isoleucine and NEFAs and between isoleucine and β-HB in both groups proposing that isoleucine could play a role in lipid metabolism. The second strongest depression of NEFAs was observed after whey intake and in particular in the prediabetic volunteers despite the greatest elevation of GIP levels whereupon GIP is known to induce lipolysis (Timper et al. 2013). However, the simultaneous increase in insulin antagonizes the effect of GIP on lipolysis (McIntosh et al. 1999). A stronger suppression of NEFA levels after whey isolate and casein intake compared to cod protein and gluten was recently reported (Holmer-Jensen et al. 2013). This observation matches with the finding in our prediabetic group that did not reveal differences between whey and casein in plasma NEFA levels. Hyperinsulinemia caused by insulin resistance could be demonstrated to elicit increased insulin action in tissues or pathways which are not as resistant as those involved in glucose metabolism (Biddinger et al. 2006) which would explain the missing differences between the protein drinks. In contrast, a difference was found in the healthy volunteers in which NEFA levels were suppressed more strongly after whey than after casein.

A whey protein drink was shown to induce the highest energy expenditure and in turn the highest fat oxidation rate when compared to a casein or soy protein drink containing carbohydrates (Acheson et al. 2011, Labayen et al. 2004, Alfenas et al. 2010). The increased fat oxidation rate was explained as a necessaty to fullfil the higher ATP demands for protein synthesis, gluconeogensis and urea cycle (Lejeune et al. 2006). Interestingly, plasma C2 acylcarnitine - which is mainly derived from acetyl-CoA from β-oxidation or from glucose metabolism, increased in concentration strongest at > 180 min after the whey drink which may be taken as an indicator of a higher fat oxidation rate after whey intake.

Glucagon was increased after all tested drinks. GMP induced the highest glucagon response and the lowest insulin level inducing the lowest NEFA response in blood over the 240 min time course. Glucagon is related to the decrease in NEFAs because strong correlations were observed between glucagon and NEFAs in our study. Plasma NEFAs are low in the presence of glucagon (Wu et al. 1990), supporting the idea that glucagon is involved in the decrease of NEFAs.

Glucagon is not able to induce lipolysis in adipose tissue. However, it seems to be able to act on hepatic lipid storage because a high protein diet was shown to reduce intrahepatocellular lipids (Bortolotti et al. 2009). In addition, glucagon is responsible for distribution of NEFAs into pathways of oxidation mainly in liver (Keller et al. 1979) with increased ketogenesis that is usually suppressed upon an increase in plasma insulin. Although whey and casein caused a major increase in insulin, their consumption did not significantly diminish plasma ß-HB levels suggesting in first place that glucagon may counteract on ketogenesis by maintaining hepatic ß-HB production. MD19 produced the expected suppression of plasma ß-HB by its effect on insulin but lack an effect on glucagon. GMP caused also a strong decrease in β-HB levels although it affected glucagon and should therefore have produced similar effects as whey and casein. This brings another possible orign of ß-HB into play. Phenylalanine and tyrosine can be metabolized to acetoacetate and in turn to β-HB (Weinhouse & Millington 1949); particular when the citric acid cycle is overloaded. Additionally, a high concentration of leucine has been demonstrated to cause the formation of ketone bodies across the hepatic bed (Abumrad et al. 1982) and this may happen at equivalent rate to their production from free fatty acids (Lund et al. 1978). This strongly suggests that in particular the high intake of phenylalanine and tyrosine as well as leucine with whey and casein produces ß-HB from their degradation in liver while this is not taking place when GMP which is free of aromatic amino acids or MD19 alone are consumed. Whereas insulin normally suppresses hepatic ketogenesis - as shown for MD19 - the need to oxidize the amino acids under conditions of high intake in the presence of glucose causes ketogenesis in particular since citric acid cycle already operates at highest activity level and a high production of $NADH_2$ in addition shifts acetoacetate:ß-HB ratio towards the latter.

5.4.5 Short chain acylcarnitines as markers of BCAA catabolism

The short chain acylcarnitines C3, C4 and C5 were strongly elevated in plasma after ingestion of the proteins. The strong correlations observed between these products and plasma BCAAs suggest that they are primarily a product of BCAA degradation (Newgard 2012). Plasma levels of free carnitine (C0) also increased slightly after all test drinks. Acetyl-carnitine (C2) derived from acetyl-CoA showed a strong suppression as a consequence of insulin action and inhibition of ß-oxidation. The decrease in C2 acylcarnitine levels was strongest after GMP despite the low prevailing insulin levels. The strong increase in C3 acylcarnitines after GMP in both volunteer groups likely reflects the catabolism of valine, isoleucine and threonine which display highly elevated plasma concentrations. C4 acylcarnitines were as well elevated after all proteins and correlations with valine concentrations suggest it to be primarily derived from valine degradation. C5 acylcarnitines were highly elevated in particular after whey intake and its levels correlated best with leucine levels. Since BCAAs have received a lot of attention by controversial findings on their role in the development of insulin resistance and/or beneficial effects on glucose disposal and as plasma markers of insulin resistance and

diabetes, the demonstration that distinct acylcarnitines in plasma mark BCAA degradation pathways may help to better define BCAA utilization in health and disease.

6 Conclusions

The experimental work demonstrates that ingestion of 50g of a whey protein isolate or a sodium caseinate together with 50g carbohydrates cause similar metabolic responses in healthy and prediabetic subjects. Postprandial blood glucose levels are lowered accompanied by high prevailing insulin levels and the effects of the proteins are more apparant in prediabetic volunteers. We propose that the two proteins alter postprandial glucose levels by diminishing the clearance rate of insulin from plasma by a yet unknown mechanism and by an increase in glucose disposal associated with the high intake of BCAAs. As a novel finding, we also registered subtle differences in the kinetics of amino acid appearance in plasma between the two groups which remain unexplained.

In our design and in both volunteer groups, we did not find any evidence for a marked difference in gastric and intestinal handling of the two different proteins nor in the kinetics of amino acid appearance in plasma arguing also for similar digestion and absorption of the protein constituents. No differences could be observed in postprandial glycemia, hormone responses, or metabolic markers such as NEFAs, β-HB or acylcarnitines between whey and casein. The feeling of hunger after intake of the whey- or casein-based test drinks was also not different. Differences in the plasma amino acid profiles were dependent on the amount of the corresponding amino acid ingested with the protein. We propose that the key reason that we could not demonstrate a difference between the two proteins – in contrast to previous studies - is that we used sodium-caseinate rather than micellar casein. Sodium caseinate shows a higher solubility and its gastrointestinal handling is thus more similar to whey protein.

The studies with glycomacropeptide produced novel results. Despite the fact, that the ingestion of glycomacropeptide reduced postprandial glycemia similar to that of whey and casein, plasma insulin response was much lower in both volunteer groups. Plasma isoleucine levels were remarkably elevated after GMP intake and isoleucine effects in promoting glucose uptake into tissues – independent of insulin - have been demonstrated. Although insulin response was smallest, the decrease of NEFA levels was strongest in both volunteer groups which would argue for higher insulin sensitivity. The lack of aromatic amino acids in GMP also revealed in comparison to the other proteins some new interdependencies between plasma amino acids and their catabolic products. Threonine, valine, isoleucine and α-aminobutyrate levels were strongly elevated after GMP intake and strong cross-correlations between them were found. Robust correlations were also found between plasma BCAA levels and concentrations of C3, C4 and C5 acylcarnitines suggesting that they can serve as metabolic markers for amino acid oxidation.

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Appendix

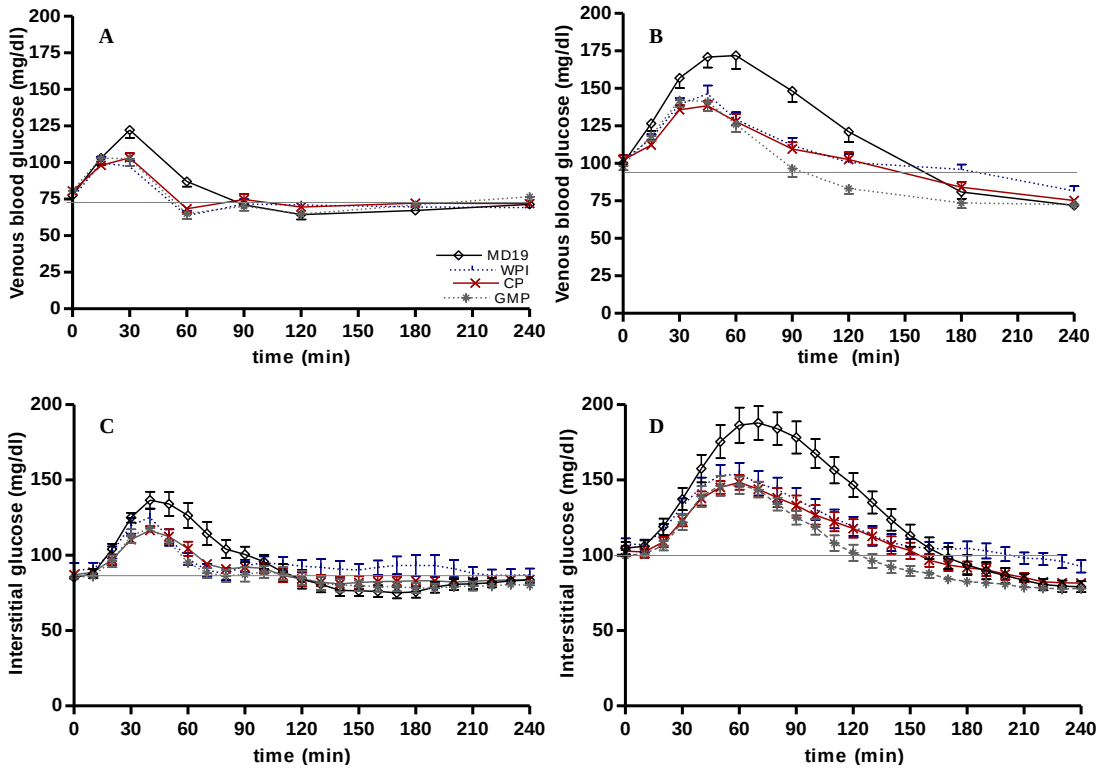


Figure 37: The effects of the different test drinks on venous blood glucose and interstitial glucose expressed in absolute values after 50g maltodextrin19 or either 50g protein or peptide ingestion (whey protein isolate, casein protein, GMP) together with 50g MD19 in healthy (**A:** n=15 **C:** n=7) and prediabetic subjects (**B:** n=15, **D:** n=14-15) on Data are expressed in mg/dl over 240 min ± SEM of venous blood glucose concentrations (A, B) and interstitial glucose (C, D). Prediabetic people were defined after WHO criteria. Significance of diets: Healthy group (A): WPI compared to MD19: $P = 0.0358$, Prediabetic group (B): WPI, CP, GMP compared to MD19 ($P = 0.0001$, $P < 0.0001$, $P < 0.0001$), WPI compared to GMP: $P = 0.0205$. Significance was analyzed by two way repeated-measures ANOVA with treatment effects and treatment x time interactions and Tukey Kramer post hoc test.

Table 17: Anthropometric measurements, BMR and RQ of healthy subjects

Subject	Age	Height	Weight	BMI	Waist	Hip	Waist/Hip	BMR	RQ
Nr.	y	m	kg	kg/m^2	cm	cm		kcal	
1	27	1.80	83	25.6	93	109	0.85	1976	0.86
2	28	1.81	83.1	25.4	90	104	0.87	1826	0.78
3	25	1.81	71.6	21.9	84	102	0.82	1628	0.85
4	27	1.81	83.8	25.6	88.5	108	0.82	2028	0.84
5	24	1.75	69.2	22.6	78	99	0.79	1862	-
6	28	1.78	71	22.4	87	100	0.87	1574	0.88
7	27	1.81	70	21.4	75	98	0.77	1936	0.79
8	29	1.84	87.4	25.8	93	108	0.86	2120	0.72
9	26	1.81	75.3	23	89	101	0.88	1744	0.91
10	27	1.76	77.5	25	86	102	0.84	1916	0.86
11	21	1.92	79.8	21.6	75	99	0.76	1852	0.81
12	24	1.80	85.8	26.5	92	105	0.88	1980	0.82
13	31	1.95	87	22.9	90	103	0.87	2144	0.86
14	28	1.99	88.9	22.4	87	105	0.83	2030	0.82
15	23	1.77	85	27.1	92	109	0.84	1964	0.83
min	21	1.75	69.2	21.4	75	98	0.77	1574	0.72
max	31	1.99	88.9	27.1	93	109	0.88	2144	0.91
x	26	1.83	79.9	23.9	86.6	103.5	0.83	1905	0.83
SD	2.55	0.07	6.92	1.95	6.11	3.78	0.04	163	0.05

Table 18: Anthropometric measurements, BMR and RQ of prediabetic subjects

Subject	Age	Sex	Height	Weight	BMI	Waist	Hip	BMR	RQ	OGTT 0 min	OGTT 120 min	HOMA*	HBA_{1c}
Nr.	**y**		**m**	**kg**	**kg/m^2**	**cm**	**cm**	**kcal**		**mg/dl**	**mg/dl**		**%**
1	63	m	1.61	77.3	29.8	101.0	103.0	1580	0.87	124	166	1.56	-
2	53	m	1.75	79.7	26.0	97.0	98.0	1792	0.87	106	182	8.04	-
3	59	m	1.91	89.6	24.6	98.0	106.0	-	-	129	167	0.36	-
4	66	m	1.76	129.9	41.9	139.0	125.0	2298	0.85	102	109	2.91	-
5	57	f	1.60	101.7	39.7	124.0	127.0	1644	0.77	104	115	4.68	-
6	68	m	1.63	72.0	27.1	97.0	102.0	1402	0.83	105	134	0.63	-
7	61	m	1.78	89.2	28.2	113.0	103.0	1710	0.82	126	214	1.57	-
8	65	m	1.92	77.8	21.1	87.0	101.0	1822	0.95	104	163	0.47	-
9	53	m	1.81	116.9	35.7	120.0	115.0	2502	0.81	96	148	2.34	-
10	64	f	1.57	73.2	29.7	92.0	106.0	1310	0.90	96	142	0.98	-
11	71	f	1.58	67.0	26.8	94.0	115.0	1304	0.80	117	162	2.07	6.1
12	67	f	1.70	81.7	28.3	84.0	104.0	-	-	106	126	0.66	6.5
13	51	m	1.78	71.0	22.4	88.5	100.0	-	-	107	181	0.65	5.9
14	72	f	1.60	66.3	25.9	98.5	108.5	-	-	113	143	0.60	6.1
15	61	m	1.81	92.0	28.1	108.5	112.0	-	-	109	129	1.36	5.6
min	51		1.57	66.3	21.1	87.0	98.0	1304	0.77	96	109	0.36	5.6
max	72		1.92	129.9	42.0	139.0	127.0	2502	0.95	129	214	8.04	6.5
x (m)	62	m	1.72	85.7	29.0	104.1	108.7	1736	0.85	110	152	1.93	6.0
SD	6.50		0.12	18.4	5.38	15.0	9.1	398	0.05	10	28	2.05	0.3

* HOMA was calculated from the challenge with 50g MD19

Table 19: Control group: Average values of measured parameters after intake of different test drinks

WPI time (min)	Glucose mg/dl	Insulin pg/ml	C-Peptide pg/ml	GLP-1 pg/ml	GIP pg/ml	Glucagon pg/ml	Ghrelin pg/ml	β-HB mg/l	NEFA nmol/l	Glycerol mg/l	H_2 ppm	VAS cm
0	75.49±1.75	136.26±26.38	1069.01±130.54	48.06±14.58	22.63±2.43	93.44±16.05	57.92±7.85	5.17±1.02	0.47±0.03	5.93±0.5	8.53±1.45	5.77±0.63
15	99.73±4.37	1095.15±215.86	3067.78±339.36	100.17±17.71	171.69±17.22	114.24±15.3	45.7±4.59	3.89±0.65	0.42±0.03	6.42±0.87	-	
30	97.26±3.88	1890.69±325.91	4609.45±641.52	88.96±14.51	221.81±20.81	136.37±15.8	34.71±4.28	3.4±0.37	0.33±0.03	4.11±0.53	5.87±1.02	
60	63.61±3.41	771.74±140.49	3398.78±221.58	79.76±16.73	199.42±30.57	167.28±25.57	32.69±3.87	3.38±0.39	0.2±0.02	4.19±0.47	2.93±0.82	3.79±0.66
90	71.5±3.46	751.07±160.12	3091.9±226.58	78.63±15.54	203.28±25.57	173.26±29.32	32.83±4.45	3.02±0.26	0.17±0.01	4.36±0.85	2.6±1.35	
120	70.87±2.85	739.99±147.84	2964.53±253.2	78.83±13.73	160.97±16.27	173.3±27.84	34.9±5.23	3.32±0.38	0.15±0.01	4.77±0.77	7.13±4.26	5.05±0.68
180	69.58±2.16	331.86±104.98	1921.19±300.91	65.8±14.66	73.83±9.36	135.18±12.79	40.76±4.54	2.98±0.33	0.24±0.03	3.78±0.54	24.27±8.23	6.95±0.59
240	69.15±1.71	159.77±61.85	1144.53±161.96	64.18±13.53	38.12±5.66	102.45±14.75	55.08±7.46	5.16±1.1	0.39±0.05	6.33±1.2	39.07±9.66	7.73±0.61
CP time (min)	**Glucose mg/dl**	**Insulin pg/ml**	**C-Peptide pg/ml**	**GLP-1 pg/ml**	**GIP pg/ml**	**Glucagon pg/ml**	**Ghrelin pg/ml**	**β-HB mg/l**	**NEFA nmol/l**	**Glycerol mg/l**	**H2 ppm**	**VAS cm**
0	80.65±1.9	159.23±32.34	1229.88±117.59	58.83±14.21	29.94±3.55	92.12±18.24	52.98±7.04	5.66±1.94	0.44±0.05	5.7±0.59	7.67±1.3	4.28±0.6
15	98.01±2.71	1000.04±158.9	2816.89±199.26	106.44±18.9	197.46±30.72	136.54±23.93	38.46±5.6	4.28±1.13	0.42±0.04	4.88±0.58	-	
30	103.29±3.09	1632.6±229.92	3852.29±251.1	101.78±14.81	218.86±28.12	133.55±22.56	32.97±4.26	3.72±0.66	0.32±0.03	5.67±0.94	6.73±1.4	
60	68.37±2.02	978.62±182.66	3569.96±290.63	97.87±15.18	212.79±32.68	177.35±30.44	32.41±3.59	3.27±0.46	0.2±0.02	5.23±0.55	4.73±1.19	2.99±0.56
90	74.78±3.88	807.06±186.15	3188.56±250.39	84.38±13.21	200.98±24.68	152.53±19.7	34.5±5.1	2.66±0.25	0.17±0.02	5.63±0.68	4.47±1.26	
120	69.51±2.53	412.48±100.23	2524.99±228.06	76.12±13.15	134.65±15.13	168.97±31.22	41.81±6.37	2.25±0.3	0.17±0.02	5.27±0.76	12±3.57	4.52±0.68
180	72.21±1.9	197.08±53.84	1619.33±170.3	60.43±13.13	64.84±5.27	130.75±16.06	44.35±5.76	2.67±0.4	0.28±0.03	5.27±0.72	31.27±9.14	6.19±0.6
240	71.89±1.66	128.02±33.12	1108.7±128.76	77.92±15.19	30.34±3.19	95.73±14.33	54.76±7.12	4.01±0.93	0.42±0.05	7.78±0.55	34.47±7.43	7.77±0.47
GMP time (min)	**Glucose mg/dl**	**Insulin pg/ml**	**C-Peptide pg/ml**	**GLP-1 pg/ml**	**GIP pg/ml**	**Glucagon pg/ml**	**Ghrelin pg/ml**	**β-HB mg/l**	**NEFA nmol/l**	**Glycerol mg/l**	**H2 ppm**	**VAS cm**
0	80.75±2.11	220.71±43.28	1346.29±137.91	45.25±5.89	28.04±2.74	61.34±12.42	55.94±7.23	2.61±0.54	0.44±0.05	7.74±0.94	10.13±2.2	5.12±0.58
15	102.99±2.72	950.04±169.24	3380.72±241.31	80.62±10.72	166.04±16.02	92.86±14.85	46.07±4.85	2.06±0.43	0.36±0.04	7.08±0.86	-	
30	102.62±4.86	1824.88±389.37	4923.3±344.47	54.21±7.17	169.36±14.11	82.89±16.51	27.74±4.07	1.4±0.19	0.25±0.03	5.3±0.53	7.8±1.2	
60	64.83±3.35	731.42±141.06	3593.23±236.03	49.32±5.11	110.77±10.28	119.69±17.47	24.49±2.15	0.84±0.12	0.12±0.01	4.36±0.43	6.27±1.05	3.03±0.52
90	70.16±3.04	622.5±122.94	3618.63±311.36	49.43±6.7	160.08±16.26	128.47±21.05	29.15±3.18	0.53±0.13	0.1±0.01	7.5±1.35	8.87±1.43	
120	64.79±2.59	464.32±112.77	2785.64±337.14	42.52±4.56	115.5±14.26	127.87±21.97	37.51±3.79	0.5±0.13	0.09±0.01	4.52±0.59	20.73±3.61	5.06±0.69
180	70.86±2.27	234.2±53.49	1637.81±244.91	46.58±6.99	42.79±8.01	92.06±15.45	54.68±8.14	1.23±0.28	0.2±0.03	7.2±0.75	38.87±10.78	6.17±0.62
240	76.59±2.19	173.42±36.7	1055.99±153.31	52.7±6.23	19.8±2.6	75.7±12.26	60.45±9.59	2.54±0.51	0.38±0.04	7.71±0.72	38.47±7.72	8.02±0.47
MD19 time (min)	**Glucose mg/dl**	**Insulin pg/ml**	**C-Peptide pg/ml**	**GLP-1 pg/ml**	**GIP pg/ml**	**Glucagon pg/ml**	**Ghrelin pg/ml**	**β-HB mg/l**	**NEFA nmol/l**	**Glycerol mg/l**	**H_2 ppm**	**VAS cm**
0	77.54±1.78	142.58±29.96	1088.96±123.67	55.43±14.65	27.98±3.65	100.52±13.79	61.9±7.17	5.93±2.17	0.48±0.05	7.19±0.93	8.4±1.41	5.12±0.72
15	103.09±4.65	732.94±114.56	2520.07±162.27	100.31±16.07	153.04±17.94	68.92±10.9	45.14±5.1	4.75±1.66	0.44±0.04	4.84±0.76	-	
30	122.21±5.26	1164.17±202.1	3623.18±237.72	87.03±12.94	169.73±21.06	71.49±11.93	38.75±5.44	2.84±0.92	0.33±0.03	5.32±0.54	7.27±1.27	
60	87.17±3.53	725.89±147.73	3679.85±274.33	72.27±13.59	150.03±21.05	73.7±10.5	32.3±3.95	1.57±0.41	0.22±0.02	3.69±0.38	6.13±1.55	4.72±0.57
90	71.04±2.59	441.58±136	2726.8±212.95	61.3±14.54	124.61±16.48	95.68±16.19	41.85±5.44	1.12±0.18	0.19±0.02	4.1±0.64	8.6±2.4	
120	64.39±3.26	274.3±106.43	1957.88±225.1	66.05±14.66	63.04±6.64	83.5±8.43	45.56±5.6	1.17±0.26	0.21±0.02	5.24±0.73	24.33±8.11	5.97±0.52
180	67.18±1.62	127.31±38.77	1108.54±114.53	60.03±14.96	23.03±2.18	103.22±12.68	54.95±6.2	3.99±1.2	0.38±0.05	5.23±0.77	30.87±6.42	7.99±0.44
240	71.43±1.19	130.07±30.85	913.22±108.54	63.46±14.25	18.64±2.65	66.81±9.1	54.01±7.74	7.28±2.23	0.51±0.06	7.44±0.73	28.07±3.36	8.43±0.48

Table 20: Prediabetic group: Average values of measured parameters after intake of different test drinks

WPI time (min)	Glucose mg/dl	Insulin pg/ml	C-peptide pg/ml	GLP-1 pmol/l	GIP pg/ml	Glucagon pg/ml	Ghrelin pg/ml	β-HB mg/l	NEFA nmol/l	Glycerol mg/l	Acetaminophen mg/ml	VAS cm	H_2 ppm
0	101.8±2.84	305.07±98.5	1240.19±129.08	1.14±0.18	161.95±22.68	124.84±23.52	328.52±81.32	5.58±1.99	0.49±0.06	10.13±1.27	0±0	1.82±0.51	5.2±0.68
15	116.47±2.93	1332.04±303.3	2123.15±234.24	5.77±0.95	680.13±90.18	194.11±35.76	309.14±74.69	5.08±1.64	0.5±0.05	10.03±1.43	7.23±1.75	-	-
30	139.27±4.4	3394.77±914.19	3396.72±336.28	7.37±0.81	900.09±97.96	238.48±36.9	261.06±59.74	4.15±1.12	0.41±0.04	7.3±1.18	11.75±0.89	-	7.07±1.23
45	146.33±5.59	5393.11±1334.16	4184.1±430.35	6.94±0.85	969.5±113.27	249.45±42.18	246.06±56.22	3.45±0.82	0.27±0.03	7.66±0.79	13.72±1.27	-	-
60	129.2±4.81	5425.98±1404.09	4231.47±339.54	5.44±0.83	867.18±76.91	220.73±33.84	212.23±57.72	3.04±0.47	0.21±0.02	7.14±0.77	14.78±1.2	2.18±0.56	5.73±1.08
90	111.87±5.22	5041.94±1132.01	4235.14±281.82	5.56±0.62	863.98±80.22	204.96±26.95	224.08±58.76	2.59±0.26	0.14±0.02	8.57±1.8	16.99±1.28	-	5.33±1.14
120	100.67±5.53	3692.25±950.03	3911.98±371.26	5.69±0.74	763.61±84.46	240.48±37.59	237.87±56.96	2.49±0.34	0.1±0.01	7.59±1.06	18.85±1.36	3.21±0.53	7.47±1.95
180	95.87±3.3	2398.02±679.03	3186.91±285.57	5.22±0.79	628.24±63.46	226.49±37.47	306.18±86.01	2.82±0.73	0.13±0.02	6.13±1	18.74±1.46	3.77±0.66	17.2±4.7
240	81.67±3.05	1026.79±286.77	2164.51±221.48	2.99±0.61	347.79±35.14	182.65±30.26	313.09±70.73	3.21±1.43	0.21±0.04	7.48±0.79	15.54±1.54	4.75±0.79	29.73±6.9
CP time (min)	**Glucose mg/dl**	**Insulin pg/ml**	**C-peptide pg/ml**	**GLP-1 pmol/l**	**GIP pg/ml**	**Glucagon pg/ml**	**Ghrelin pg/ml**	**β-HB mg/l**	**NEFA nmol/l**	**Glycerol mg/l**	**Acetaminophen mg/ml**	**VAS cm**	**H2 ppm**
0	102.2±3.23	393.77±112.4	1307.26±158.21	1.14±0.18	163.53±21.21	113.56±19.51	341.85±89.83	4.28±0.93	0.52±0.05	10.13±1.3	0±0	2.83±0.61	6.33±1.65
15	112.27±3.43	788.48±149.63	1779.33±193.8	3.54±0.50	546.86±70.82	149.46±24.41	334.69±69.53	4.55±1.22	0.5±0.05	9.19±1.19	5.34±0.98	-	-
30	135.73±3.25	2343.76±567.9	3124.52±346.06	6.96±1.01	922.98±71.23	248.7±51.49	281.65±63.59	4.29±1.07	0.45±0.04	9.31±1.07	11.09±0.83	-	6.27±1.54
45	138.4±3.25	3900.64±1524.23	3694.51±363.45	5.76±0.57	895.89±82.05	233.29±46.28	260.43±60.53	3.58±0.6	0.33±0.04	8.87±1.36	14.03±1.07	-	-
60	127.87±5.21	3473.18±670.51	4103.42±360.19	5.34±0.69	827.88±69.37	202.34±29.98	240.66±54.63	3.19±0.41	0.23±0.02	8.86±0.89	15.08±1	1.84±0.44	4.87±1.34
90	109.6±4.4	3093.83±452.85	4434.85±394.58	5.03±0.79	867.23±59.99	206.82±32.81	224.98±52.59	2.64±0.41	0.14±0.01	7.98±1.11	18.48±0.98	-	5.07±1.29
120	102.53±4.95	2447.44±240.6	4125.77±301.67	4.30±0.53	802.02±54.21	212.44±31.58	231.63±55.52	2.28±0.34	0.11±0.01	8.52±1.17	21.05±1.19	2.68±0.63	8.4±2.6
180	84±3.82	1262.73±239.71	2777.5±213.91	2.82±0.56	519.56±45.37	204.58±33.24	323.92±71.51	2.29±0.29	0.15±0.02	7.51±0.64	18.65±1.67	3.73±0.78	25±7.11
240	75.2±2.64	492.25±107.96	1982.48±273.13	2.22±0.64	289.54±29.77	158.62±24.48	353.56±90.2	2.91±0.57	0.27±0.03	9.95±0.84	14.82±1.71	4.7±0.83	29.07±7.09
GMP time (min)	**Glucose mg/dl**	**Insulin pg/ml**	**C-peptide pg/ml**	**GLP-1 pmol/l**	**GIP pg/ml**	**Glucagon pg/ml**	**Ghrelin pg/ml**	**β-HB mg/l**	**NEFA nmol/l**	**Glycerol mg/l**	**Acetaminophen mg/ml**	**VAS cm**	**H2 ppm**
0	98.47±2.96	384.89±99.48	1257.3±133.82	1.14±0.18	202.78±28	121.57±17	399.37±102.63	4.31±1.43	0.51±0.05	10.13±1.15	0±0	2.43±0.63	12.93±5.35
15	118.27±3.47	1506.85±497.57	2087.05±290.35	5.70±1.08	771.2±91.6	189.12±32.88	409.93±86.5	4.12±1.39	0.46±0.03	9.58±0.97	7.96±1.41	-	-
30	142.13±4.26	2817.81±755.87	3166.57±355.71	7.58±0.92	878.9±72.99	226.35±35.53	359.91±83.77	3.45±1.11	0.39±0.03	8.76±0.99	13.47±1.17	-	9.73±2.28
45	141.07±6.04	2831.29±498.75	3842.66±401.15	6.68±0.87	830.59±74.38	225.05±34.7	308.62±77.72	2.7±0.68	0.27±0.03	8.19±0.91	16.22±0.93	-	-
60	125.87±4.85	2990.94±562.2	4019.38±295.14	5.09±0.51	807.1±62.05	215.32±33.09	316.02±77.45	2.11±0.45	0.19±0.02	8.18±0.96	18.68±1.12	2.78±0.43	6.8±1.63
90	96.53±5.35	2342.87±260.62	3899.09±288.31	5.23±0.54	763.15±44.83	213.43±29.16	283.25±80.08	1.51±0.28	0.12±0.01	7.01±0.54	22.4±1.21	-	9.27±2.31
120	83.2±3.35	1726.04±254.9	3305.12±234.27	4.35±0.49	716.64±44.57	230.24±29.99	319.29±81.02	1.51±0.24	0.1±0.01	7.32±0.99	24.81±1.7	3.56±0.4	17.07±5.38
180	73.53±3.33	900.79±249.55	2161.84±217.47	2.59±0.36	390.56±35.17	162.74±21.7	390.3±88.26	1.39±0.22	0.16±0.03	8.37±1.01	21.5±1.92	5.59±0.53	40.2±10.01
240	72.47±2.36	350.1±106.93	1359.52±125.8	1.93±0.36	210.88±16.31	144.29±18.53	444.76±89.44	3.52±0.88	0.39±0.05	12.69±2.53	15.46±1.35	7.92±0.36	58.33±12.96
MD19 time (min)	**Glucose mg/dl**	**Insulin pg/ml**	**C-peptide pg/ml**	**GLP-1 pmol/l**	**GIP pg/ml**	**Glucagon pg/ml**	**Ghrelin pg/ml**	**β-HB mg/l**	**NEFA nmol/l**	**Glycerol mg/l**	**Acetaminophen mg/ml**	**VAS cm**	**H2 ppm**
0	100.13±2.94	331.99±92.04	1285.06±117.94	1.14±0.18	222.77±70.38	115.87±19.42	366.58±97.65	4.77±1.03	0.54±0.05	10.69±1.01	0±0	2.47±0.63	4.73±0.98
15	126.67±4.92	885.76±190.15	1905.84±221.18	5.34±0.91	666.75±84.33	112.49±21.14	329.21±75.62	3.95±1.03	0.54±0.05	10.41±0.86	14.31±1.84	-	-
30	156.87±6.58	1509.36±313.78	2582.3±292.57	6.45±0.69	795.41±89.56	116.3±21.86	339.46±79.54	3.62±0.89	0.44±0.04	9.08±1.47	18.51±1.72	-	7.2±1.92
45	170.87±7.01	2748.65±938.31	3459.85±371.2	6.41±0.77	785.04±79.57	115.3±18.21	275.61±69.99	2.62±0.56	0.33±0.04	8.61±1.25	23.45±1.08	-	-
60	171.93±8.89	1814.18±191.92	3535.96±230.98	4.52±0.59	721.16±73.3	105.05±11.36	253.36±42.79	1.9±0.39	0.24±0.03	9.16±1.13	24.62±1.48	2.69±0.46	6.93±1.73
90	148.27±7.36	2050.56±336.87	4093.7±217.68	3.06±0.54	639.53±51.25	101.86±15.37	346.34±109.28	1.74±0.24	0.19±0.03	9.13±1.2	27.07±1.89	-	8.53±2.88
120	121.13±6.87	1873.67±467.17	3695.16±243.53	2.06±0.47	467.38±28.17	97.6±13.16	338.09±112.19	1.35±0.27	0.16±0.04	7.89±0.78	26.37±2.19	3.99±0.59	14.47±3.59
180	80.93±4.55	574.54±111.77	2219.06±197.19	1.36±0.41	248.2±31.14	102.95±11.36	416.32±130.44	1.78±0.34	0.2±0.03	8.91±1.18	20.98±2.1	5.44±0.7	45.27±10.8
240	71.93±2.03	253.62±67.84	1464.17±112.14	0.69±0.14	165.03±18.74	107.63±17.01	467.84±129.75	3.63±0.8	0.34±0.06	9.6±1.04	16.71±1.67	6.03±0.88	50.07±12.43

Table 21: Control group: Postprandial amino acids after intake of the WPI drink

AA (µM)	0 min	15 min	30 min	60 min	90 min	120 min	180 min	240 min
Aad	1.2±0.1	1.17±0.07	1.67±0.11	3.31±0.22	4.79±0.4	5.93±0.49	5.91±0.37	3.68±0.33
Abu	26.63±1.49	27.62±1.55	31.97±1.31	32.19±1.31	30.84±1.65	28.27±1.35	24.35±0.97	21.59±1.02
Ala	320.73±17.24	363.27±13.09	455.2±18.52	522.8±23.71	522±27.51	506.93±23.8	425±23.72	384.27±17.15
Arg	82.17±3.77	107.65±5.11	127.35±6.43	125.03±6.14	124.93±5.99	119.32±5.82	87.94±5.5	72.74±2.9
Asn	67.45±2.27	90.43±3.72	115.32±5.65	119.29±5.99	114.23±5.02	108.82±4.79	80.36±3.6	66.24±2.46
Asp	2.22±0.2	3.17±0.36	6.42±0.79	6.74±0.82	6.1±0.57	7.62±1.17	3.42±0.42	2.1±0.21
Cit	33.37±1.74	32.43±1.48	32.23±1.28	39.4±1.97	41.84±2.89	48.01±3.55	45.94±1.34	39.71±1.68
Cys	205.58±5.24	238.64±7.66	292.94±9.41	315.34±8.12	315.05±7.93	298.40±9.76	264.34±5.97	209.86±5.26
EtN	33.16±9.2	30.95±9.12	40.01±14.21	31.23±10	31.49±9.21	31.68±9.23	28.05±7.72	29.58±8.94
Gln	652.73±21.05	689.53±16.04	743.2±19.85	747.67±23.76	721.6±28.45	724±26.19	667.53±25.22	673.8±18.51
Glu	34.33±4.07	43.09±5.22	50.73±5.8	56.44±5.2	50.67±5.12	56.93±6.84	40.63±3.28	29.81±4
Gly	200.87±6.69	214.53±6.69	230.13±10.33	216.73±8.32	204.53±9.32	197.13±9.47	171.07±7.27	164.73±6.49
His	86.31±2.03	91.71±2.34	104.51±2.83	104.85±2.72	100.86±3.26	96.24±3.33	86.74±3.2	81.99±1.2
Hyp	12.83±1.07	24.12±3.52	47.48±7.6	68.93±9.63	73.21±9.78	78.32±10.22	62.97±7.57	50.23±5.84
Ile	87.63±10.3	152.47±9.61	212.67±7.8	217.2±7.23	218.67±7.38	210.53±6.34	129.87±12.07	81.19±6.99
Leu	144.93±4.07	297.8±21.12	405.93±17.2	442.67±16.5	449.73±14.94	453.93±13.47	299.6±22.52	210.67±12.6
Lys	185.67±5.92	300.47±15.69	399.87±17.59	432.73±21.37	434.6±17.52	429.07±15.75	292.27±19.61	229.87±10.71
Met	22.4±3.77	36.71±6.11	49.47±9.73	47.78±7.56	44.31±6.57	45.75±6.83	29.64±4.47	21.15±2.34
Orn	50.1±2.56	61.29±2.99	66.22±3.4	68.21±3.16	67.33±3.08	65.83±3.34	58.95±3.5	48.19±2.49
PEtN	2.39±0.15	2.68±0.18	3.21±0.2	3.18±0.28	2.82±0.16	2.62±0.17	2.28±0.14	2.42±0.14
Phe	59.07±1.9	80.26±2.61	91.39±2.39	88.97±3.06	87.62±2.44	82.4±1.82	59.48±3.3	49.35±1.64
Pro	189.33±10.48	228±8.74	268.73±10.75	276.73±10.18	273.33±9.98	272.53±11.05	226.53±10.26	196.13±7.54
Ser	110.39±4.16	144.6±6.02	170.13±7.48	165.87±8.23	157.67±8.1	146.47±8.12	110.41±5.79	94.33±5.67
Tau	47.69±2.26	46.63±2.07	48.27±2.28	47.71±2.19	44.43±2.49	42.75±2.28	41.39±2.01	44.24±1.66
Thr	131.51±10.53	170.87±8.45	217.6±11.95	229±10.82	226.73±11.46	224.4±10.47	172.53±9.52	143.87±6.58
Trp	54.03±2.36	76.88±2.94	97.17±2.62	108.9±3.23	115.93±4.1	116.93±4.02	93.51±3.68	71.75±2.92
Tyr	64.43±3.67	89.31±4.07	108.85±5.14	114.74±5.39	117.85±5.41	117.48±5.05	88.58±4.27	71.55±2.64
Val	290.4±16.84	354.4±13.35	428±10.39	455.33±10.37	457±10.84	462±10.03	359.67±14.74	290.27±8.63

Table 22: Control group: Postprandial amino acids after intake of the CP drink

AA (µM)	0 min	15 min	30 min	60 min	90 min	120 min	180 min	240 min
Aad	1.11±0.07	1.19±0.07	1.38±0.09	2.26±0.16	3.08±0.14	3.15±0.15	2.81±0.16	1.99±0.13
Abu	24.74±2.32	26.63±2.72	30.2±3.16	31.58±2.8	30.99±2.65	27.72±2.08	24.53±1.92	23.39±2
Ala	370.8±24.61	398.67±28.63	500.07±32.77	588.93±30.82	589.53±23.98	516.4±22.79	439.33±23.39	402.07±19.29
Arg	80.18±4.53	112.69±6.58	134.49±8.6	137.04±7.66	130.25±6.52	117.29±6.65	95.2±4.57	78.55±2.86
Asn	64.45±2.14	87.61±4.38	121.54±7.95	129.18±7.81	123.34±4.58	104.75±4.22	83.07±3.61	70.07±2.59
Asp	2.4±0.18	2.91±0.24	4.17±0.44	4.76±0.63	5.4±0.89	3.39±0.36	3±0.29	2.31±0.19
Cit	30.63±1.64	32.31±1.96	32.85±1.99	35.97±2.01	41.42±2.12	46.17±3.31	48.1±2.32	40.21±1.88
Cys	211.90±6.74	208.38±6.89	234.84±10.05	233.34±8.59	226.82±4.93	215.46±4.72	197.04±4.72	195.34±6.05
EtN	23.6±7.73	30.79±12.42	33.04±13.06	31.64±13.63	34.43±13.56	29.89±10.41	25.84±7.93	25.53±8.6
Gln	642.6±19.37	641.4±21.05	727.47±32.16	756.93±33.32	742±23.96	698.13±26.78	684.07±27.37	670.2±19.02
Glu	37.57±3.98	47.08±4.47	54.91±4.86	54.94±6.02	50.47±5.23	49.84±5.01	41.67±5.39	27.86±3.62
Gly	211±10.37	220.87±9.76	251.87±11.14	246.73±10.36	243.33±10.54	218±10.94	194.73±9.62	191.6±8.92
His	81.53±2.98	87.75±3.45	109.45±5.54	113.66±6.12	104.67±2.02	95.5±3.02	88.34±2.85	80.69±2.1
Hyp	12.78±2.05	24.49±3.58	57.15±9.19	77.72±12.35	94.58±14.17	88.89±11.84	62.09±7.53	51.58±6.27
Ile	73.46±3.65	125.84±7.35	178.2±10.15	200.78±11.17	189.2±5.39	164.8±8.48	119.86±6.69	86.25±6.15
Leu	140.6±6.07	247.47±14.44	311.73±14.9	341.87±19.67	328.07±8.28	288.13±13.05	226.47±11	170.47±8.74
Lys	165.4±6.38	254±13.53	344.47±18.03	354.47±17.97	328.33±12.28	297.27±11.74	238.67±10.59	194.93±7.3
Met	23.31±3.53	41.69±5.55	57.82±8.51	58.95±7.3	57.33±6.71	46.12±5.04	36.96±4.28	28.3±3.79
Orn	51.77±2.59	64.68±3.68	76.36±5.08	82.45±5.46	85.28±4.57	79.98±4.39	72.11±3.99	60.31±3.69
PEtN	2.28±0.13	2.43±0.17	3.16±0.25	3.55±0.28	2.95±0.25	2.86±0.17	2.52±0.15	2.66±0.22
Phe	57.27±1.87	83.32±3.79	96.96±4.66	102.71±5.57	100.53±2.56	90.05±3.66	72.07±2.6	59.68±1.68
Pro	192.67±14.02	287.93±18.46	393.13±25.92	460.33±30.1	482.2±22.75	437.33±21.27	359.6±14	298.47±14.54
Ser	109.81±3.88	148.23±7.01	195.67±12.05	196.73±11.36	190.8±7.83	162.73±8.09	131.76±5.66	115.62±4.94
Tau	45.28±2.08	46.83±2.18	46.75±1.89	47.42±2.54	45.53±2.04	43.66±1.66	41.6±1.97	42.91±1.66
Thr	123.8±7.8	152.32±10.08	204.86±14.75	218.74±15.3	219.07±10.82	198.93±11.13	160.4±9.39	141.13±7.88
Trp	54.22±1.82	67.94±2.64	74.51±3.03	78.45±3.47	75.79±1.96	70.15±2.45	59.41±1.74	50.89±1.99
Tyr	65.71±3.39	106.89±6.96	133.87±7.49	152.07±8.67	149.87±4.82	142.51±6.63	120.87±3.64	97.03±3.91
Val	257±10.69	338.47±15.29	426.4±22.21	491.33±24.01	507.93±12.72	478.07±17.98	421.67±13.33	357.8±14.5

Table 23: Control group: Postprandial amino acids after intake of the GMP drink

AA (µM)	0 min	15 min	30 min	60 min	90 min	120 min	180 min	240 min
Aad	1.63±0.09	1.63±0.11	1.73±0.08	2.17±0.15	2.56±0.23	2.84±0.25	2.42±0.23	1.89±0.13
Abu	23.98±0.96	27.82±1.19	34.33±1.54	39.22±1.57	42.17±1.67	42.27±2.06	38.31±1.6	37.17±1.9
Ala	368.73±20.77	434±26.93	576.87±31.83	682±21.5	708.33±17.33	649.73±20.87	508.4±25.44	443.6±19.75
Arg	88.08±4.42	100.16±4.59	106.6±4.96	106.39±5.35	102.17±4.66	96.74±4.32	82.69±2.93	74.76±2.55
Asn	74.31±2.01	117.51±6.18	170.53±8.02	178.73±5.69	184.53±7.92	154.13±5.94	101.42±4.86	87.45±2.93
Asp	2.94±0.25	3.58±0.21	4.93±0.49	6.26±0.53	7.08±0.88	5.56±0.49	3.5±0.38	2.48±0.18
Cit	33.99±1.91	32.6±1.43	31.71±1.34	33.45±1.94	37.92±2.43	43.68±2.66	40.29±1.58	35.75±1.44
Cys	243.58±4.79	252.88±5.35	275.06±5.66	269.94±5.22	260.94±4.52	232.32±5.25	220.10±5.27	212.16±5.65
EtN	24.8±2.73	20.33±2.35	25±2.79	20.13±2.27	25.27±2.66	22.6±2.13	22.53±2.18	23.33±1.88
Gln	751.13±17.8	764.2±15.63	833.73±24.73	847.53±22.23	843.67±20.75	821.47±25.27	747.8±19.51	754.33±15.53
Glu	36.82±4.46	47.26±5.37	59.56±5.9	70.27±5.89	68.82±7.21	64.15±6.27	41.32±3.51	28.54±2.81
Gly	233.8±7.73	247.6±8.51	265.27±8.99	265.87±9.14	265.47±8.83	246.53±8.43	215.27±5.53	212.93±6.28
His	94.29±3.15	97.29±2.79	100.4±2.53	96.77±2.59	93.74±2.36	88.96±2.9	87.02±2.18	88.4±2.13
Hyp	11.24±0.88	13.33±1.04	15.93±1.24	16.65±1.23	16.29±1.17	14.42±1.1	11.87±1.08	10.57±0.84
Ile	72.13±2.43	187.34±15.36	303.4±13.08	368.33±12.14	436.27±16.57	424.67±14.09	304.2±14.54	230.34±15.09
Leu	150.27±4.82	181.6±5.93	202.13±4.14	185.2±4.62	178.07±3.81	149.33±5.22	118.47±3.93	120.56±4.59
Lys	187.8±5.5	254.07±9.8	318.53±10.91	333.6±9.31	348.87±9.92	315.2±10.3	231.07±9.26	197.6±6.7
Met	26.49±3.59	43.09±6	50.24±7.05	51.2±5.96	52.8±6.61	43.3±4	27.71±1.65	27.27±2.21
Orn	52.47±2.99	61.21±3.49	73.3±3.8	68.93±3.55	68.39±3.35	64.09±3.74	55.78±1.98	50.98±2.82
PEtN	2.93±0.18	3.08±0.13	3.86±0.22	4.24±0.28	4.09±0.18	3.62±0.19	3.11±0.18	3.05±0.19
Phe	62.33±1.52	66.23±1.18	64.92±0.96	56.64±1.26	47.82±1.42	38.2±1.38	34.87±1.32	40.68±1.74
Pro	184.73±9.48	292.93±19.15	407.67±17.56	475.8±21.04	527.53±25.87	490.93±21.66	350.47±16.68	283.53±15.16
Ser	116.47±4.69	172.93±10.61	235.13±14.79	243.27±11.64	245.8±13.53	211.53±10.33	148.07±7.16	131.6±5.26
Tau	58.19±2.65	58.36±2.26	60.42±2.5	62.89±2.83	61.89±1.85	59.8±2.08	55.21±2.11	53.19±1.97
Thr	127.88±5.78	247.33±22.53	380.13±27.48	456.87±25.42	525±32.99	491.47±25.32	340.67±17.42	293.33±18.16
Trp	54.35±1.48	55.19±1.52	53.47±1.52	47.07±1.61	39.53±1.66	31.83±1.18	30.93±1.37	35.86±1.93
Tyr	71.04±3.24	72.69±2.58	71.84±2.38	62.51±2.33	53.86±2.16	44.4±1.92	38.13±1.6	39.93±1.98
Val	275.33±7.32	415.4±19.54	547.07±14.81	615.47±16.44	706.87±17.3	689.33±16.54	584.87±15.71	510.8±20.47

Table 24: Control group: Postprandial amino acids after intake of the MD19 drink

AA (µM)	0 min	15 min	30 min	60 min	90 min	120 min	180 min	240 min
Aad	1.18±0.07	1.16±0.08	1.18±0.15	1.05±0.05	0.99±0.06	0.97±0.07	1.13±0.06	1.02±0.06
Abu	25.33±1.51	26.05±1.79	26.51±1.77	23.61±1.55	22.41±1.55	20.36±1.34	21.24±1.56	21.5±1.28
Ala	344.93±21.22	332.53±21.95	355.87±20.23	359.87±17.3	365.87±16.9	357.53±15.23	337.13±15.15	326±13.19
Arg	79.01±3.95	75.73±3.83	73.84±3.76	65.49±2.98	63.63±3.08	63.07±2.96	66±2.64	70.05±2.84
Asn	64.57±2.7	63.9±3.21	70.97±3.31	65.47±2.42	63.37±2.16	61.72±2.27	60.49±1.8	62.21±1.69
Asp	2.15±0.14	2±0.15	2.03±0.13	1.79±0.1	1.85±0.21	1.86±0.14	1.97±0.31	1.98±0.2
Cit	32.04±1.84	28.54±1.48	27.23±2.27	20.88±0.92	20.07±0.97	20.29±1.09	25.35±1.32	26.97±1.4
Cys	106.01±6.33	96.34±6.48	107.87±5.53	103.28±5.02	99.05±5.44	102.78±5.07	97.23±3.68	99.47±4.66
EtN	36.78±12.23	34.54±13.31	37.86±14.78	38.85±14.87	39.57±15.05	39.01±14.27	34.63±11.35	30.95±8.95
Gln	645.53±25.78	611.67±21.29	647.07±26.13	603.67±17.94	611.8±16.24	597.13±17.27	652.93±15.53	675.13±14.24
Glu	37.59±3.37	39.69±4.31	40.81±3.27	33.78±3.1	33.07±3.32	29.87±3.56	31.64±3.78	25.91±3.66
Gly	212.47±11.55	212.47±10.55	210.87±11.54	201.27±9.55	209.67±10.16	203.8±7.9	205.4±8.67	205.27±8.46
His	80.99±2.83	79.41±3.04	79.79±2.08	78.89±2.9	74.69±2.05	74.41±2.14	79.42±1.95	81.63±2.04
Hyp	13.3±1.22	34.45±4.37	67.16±10.2	82.7±11.4	90.34±12.34	72.26±8.61	57.9±6.53	53.98±5.9
Ile	77±3.94	72.59±3.1	81.66±13.7	56.55±2.63	48.47±2.17	43.43±1.87	50.17±2.08	57.99±2.31
Leu	145.27±5.98	137.47±5.08	128.93±4.73	110.73±4.76	95.71±3.6	87.1±3.14	102.31±4.21	118.56±4.95
Lys	177.33±5.7	170.6±5.24	171.2±7.69	155.53±5.43	155±4.53	155.2±4.12	165.07±5.46	168.73±4.61
Met	21.45±2.77	21.06±2.74	20.09±2.02	17.24±2.27	15.1±2	14.66±1.99	14.84±2.16	17.67±2.82
Orn	50.37±2.37	48.91±2.52	50.43±4.51	43.53±2.43	44.01±3.06	40.71±2.23	41.55±2.75	40.33±2.4
PEtN	2.52±0.14	2.23±0.12	2.09±0.15	1.9±0.13	1.93±0.15	1.97±0.15	2.12±0.11	2.3±0.16
Phe	60.11±2.1	55.93±1.8	53.65±1.75	49.93±1.43	46.85±1.17	44.53±1.24	47.69±1.24	52.17±1.28
Pro	178.15±12.97	174.6±14.25	198.4±20.95	171.4±11.42	169.4±11.56	164.33±10.37	156.91±11.37	159.47±9.77
Ser	113.53±5.34	112.07±5.43	119.74±7.67	104.81±4.92	103.14±4.63	95.75±4.16	98.55±5.68	100.27±4.73
Tau	47.27±2.01	47.45±1.8	43.65±1.76	42.2±1.64	42.17±1.82	42.05±2	42.97±1.68	42.39±1.71
Thr	117.32±7.23	116.48±7.92	146.73±24.84	113.32±4.62	109.28±4.38	104.01±4.49	101±6.54	104.15±4.36
Trp	54.87±1.88	53.87±1.93	50.63±2.14	48.81±1.59	46.43±1.29	43.67±1.38	45.39±0.96	46.01±1.1
Tyr	63.49±3.19	59.15±2.76	56.69±2.44	51.54±2.2	46.65±1.64	44.19±1.83	46.65±1.74	49.58±1.48
Val	265.4±11.08	258±9.82	272.73±20.19	235.8±9.25	220.13±8.5	204.53±7.09	216.13±8.31	225.8±7.62

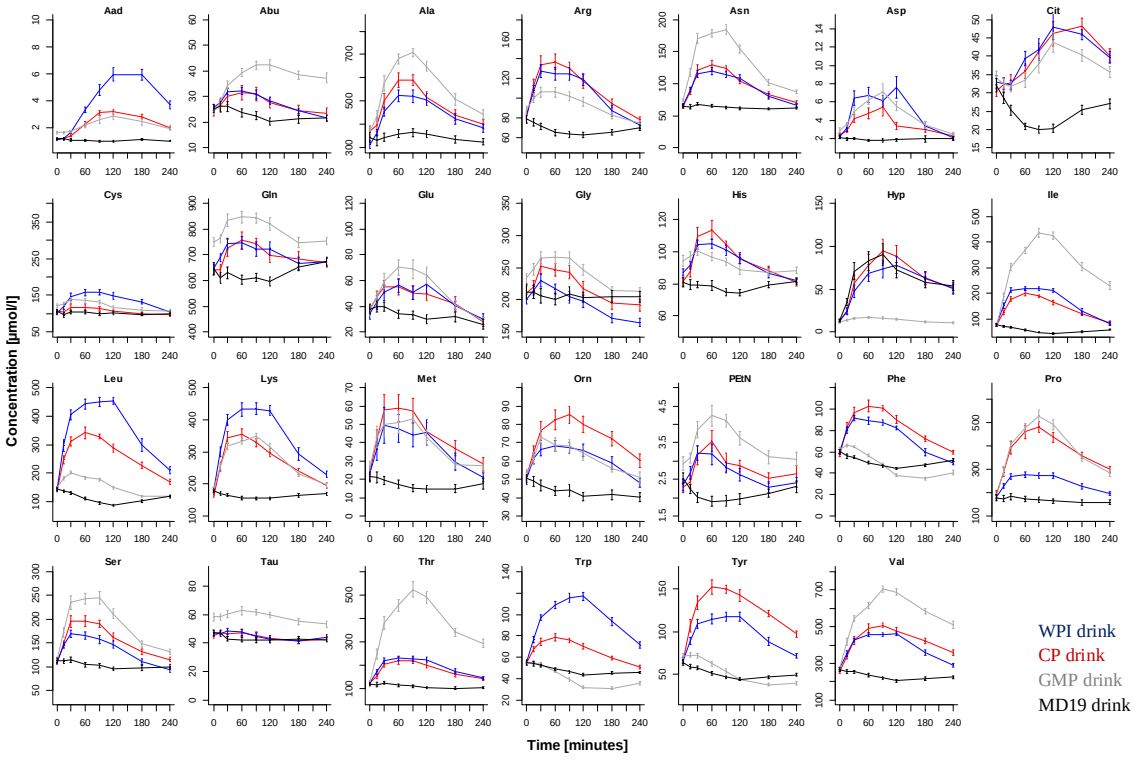


Figure 38: Postprandial amino acid response after intake of the WPI drink, CP drink, GMP drink and the MD19 drink in the healthy group.

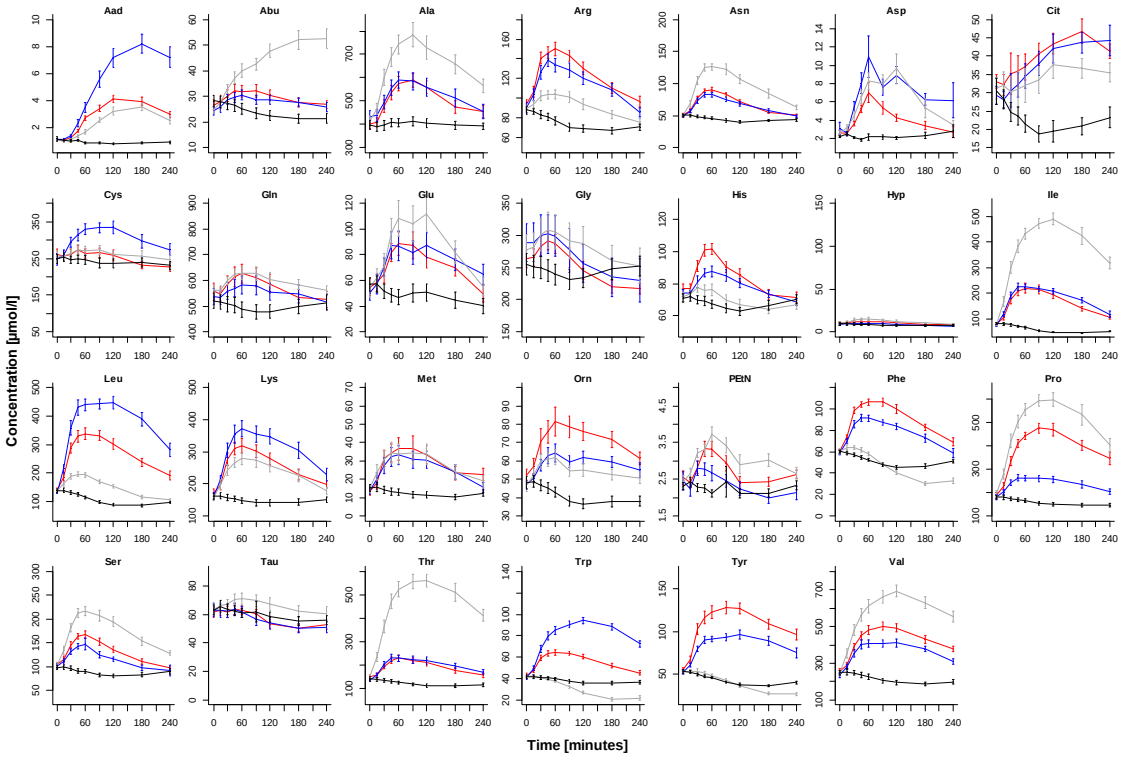


Figure 39: Postprandial amino acid response after intake of the WPI drink, CP drink, GMP drink and the MD19 drink in the prediabetic group. Hydroxyproline was not added in the test drinks.

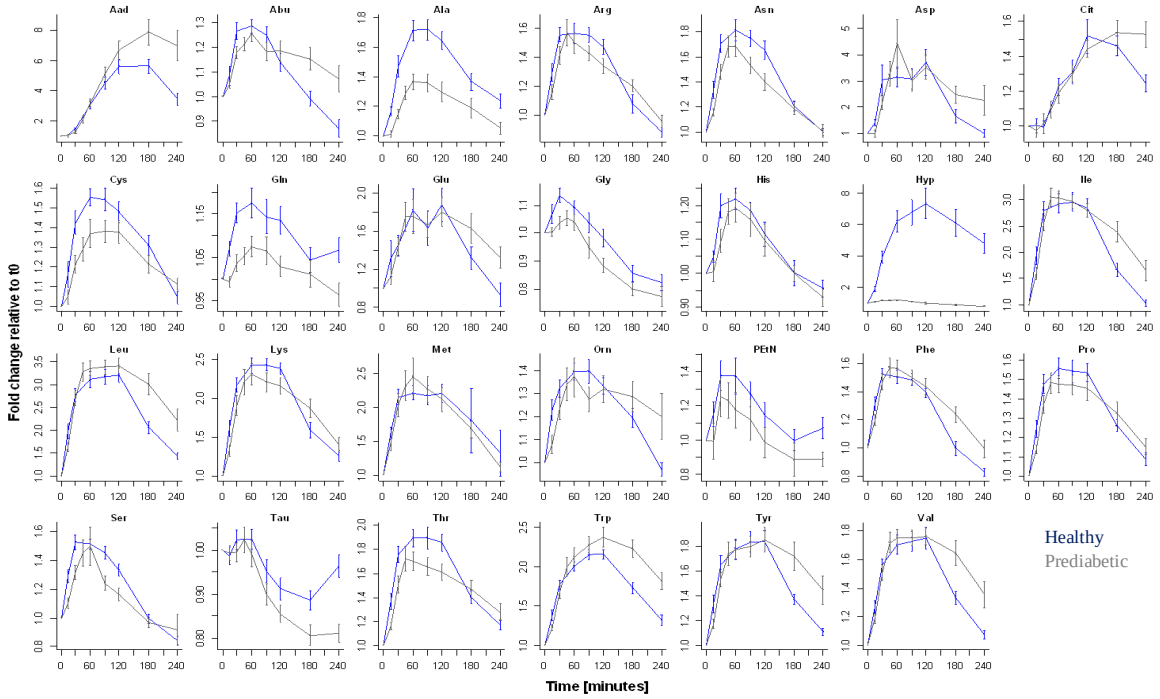


Figure 40: Comparison of amino acids between the healthy group and the prediabetic group after the WPI drink

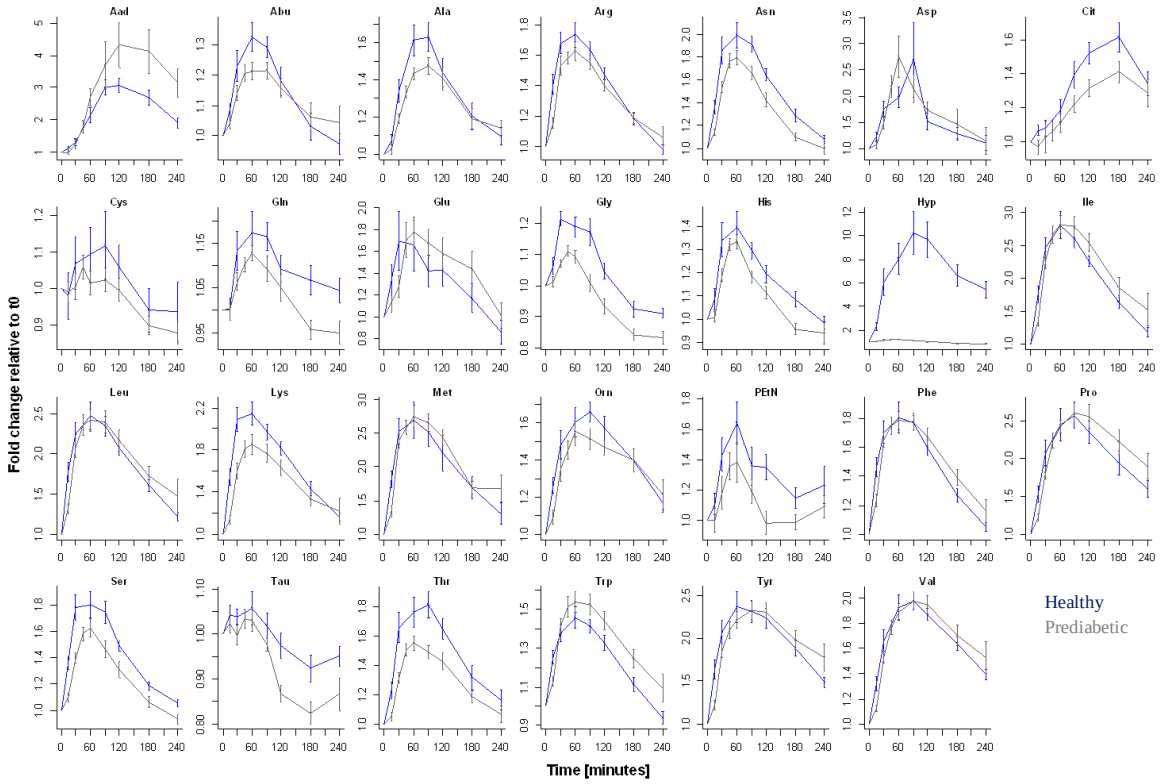


Figure 41: Comparison of amino acid drinks between the healthy group and the prediabetic group after the CP drink

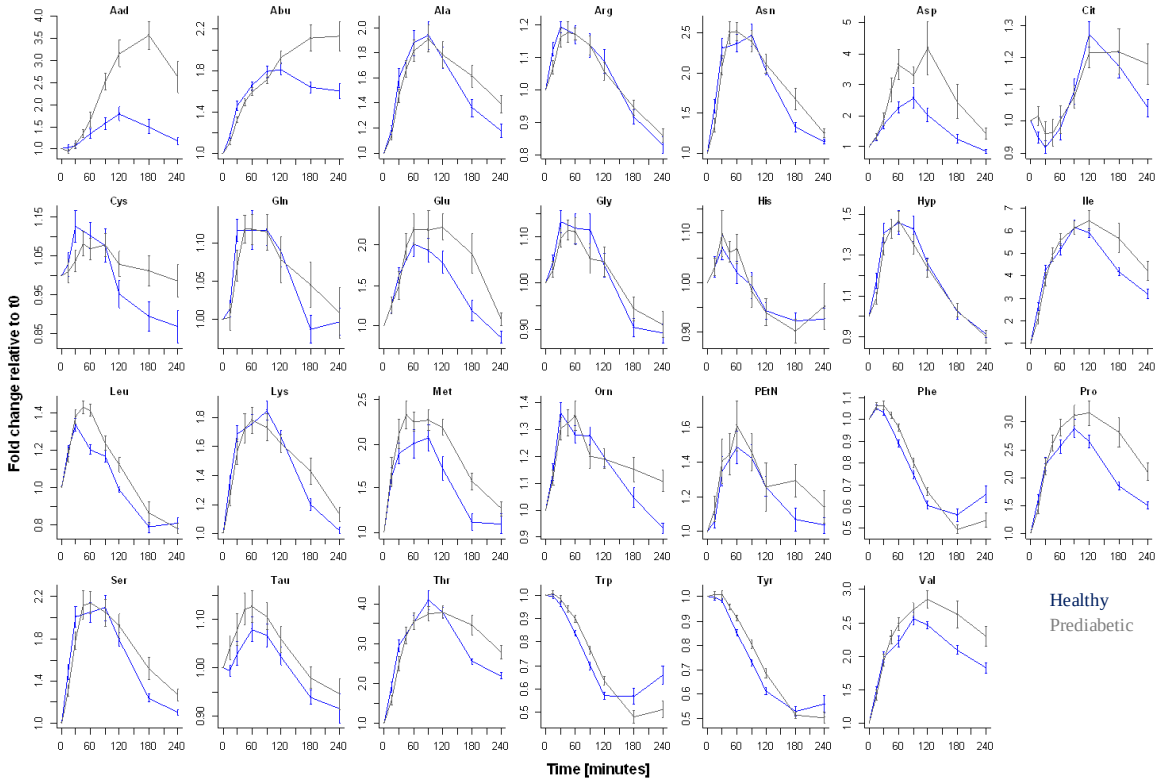


Figure 42: Comparison of amino acids between the healthy group and the prediabetic group after the GMP drink

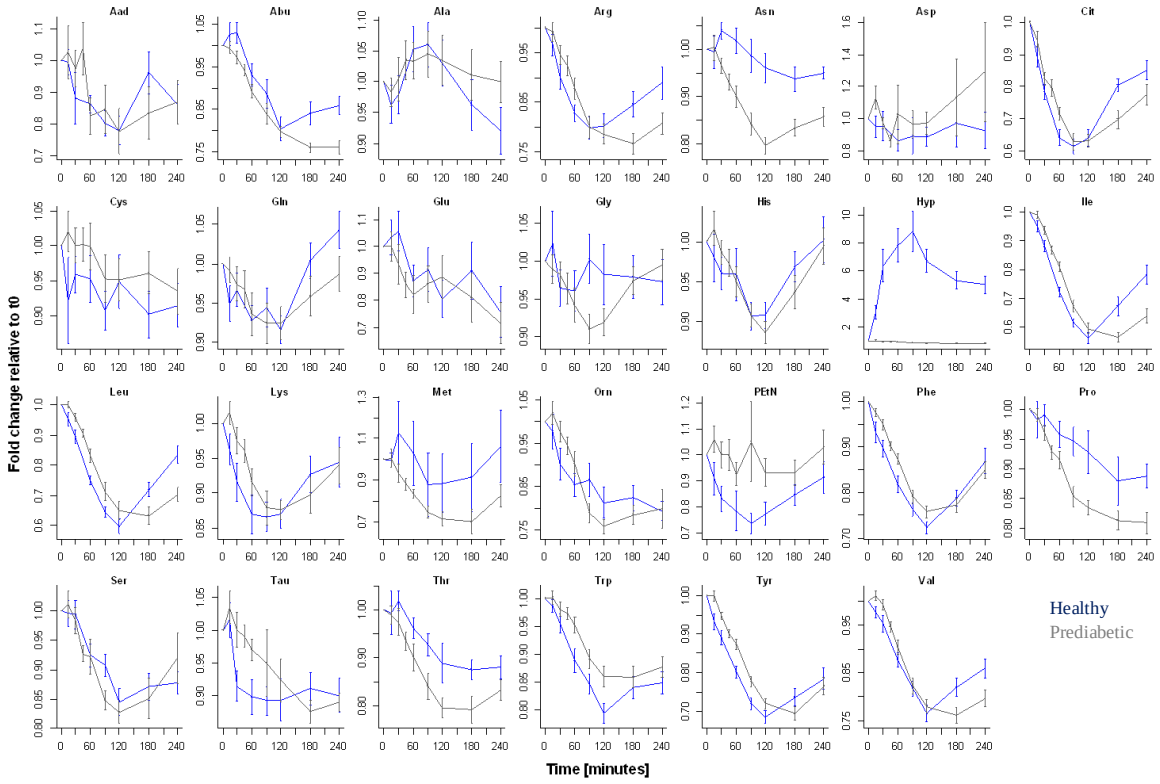


Figure 43: Comparison of amino acids between the healthy group and the prediabetic group after the MD19 drink

Table 25: Prediabetic group: Postprandial amino acids after intake of the WPI drink

AA (µM)	0 min	15 min	30 min	45 min	60 min	90 min	120 min	180 min	240 min
Aad	1.1±0.06	1.11±0.09	1.36±0.12	2.34±0.26	3.48±0.37	5.56±0.58	7.15±0.67	8.19±0.67	7.19±0.74
Abu	24.49±1.72	25.71±1.78	28.83±2.04	29.57±1.97	30.57±2.02	28.67±1.8	28.61±1.76	27.7±1.59	25.88±1.77
Ala	432.79±25.48	438.47±25.7	495.41±27.34	556.8±31.51	588.26±34.68	584.81±36.42	558.54±39.02	513.52±37.7	455.26±30.5
Arg	90.52±5	103.82±6.05	126.54±7.05	138.62±6.22	133.89±6.95	128.61±7.53	120.39±6.82	108.53±6.41	85.96±4.72
Asn	49.47±1.85	56.97±2.16	73.25±3.79	82.39±4.07	82.5±3.88	75.36±3.75	68.85±3.64	58.45±2.88	49.24±2.25
Asp	3.11±0.62	2.57±0.25	5.46±0.54	8.02±1.11	10.91±2.26	7.58±0.87	8.89±0.94	6.26±0.64	6.17±1.9
Cit	29.28±2.37	28.07±2.18	30.56±4.52	32.19±3.3	34.46±3.32	37.95±3.37	42.12±3.91	43.68±2.77	44.29±4.11
Cys	248.24±15.58	258.89±15.66	294.96±13.66	313.95±16.1	329.78±14.56	333.68±13.02	335.35±17.52	297.54±18.72	273.89±16.03
EtN	9.31±0.85	8.96±0.75	8.7±0.83	9.31±0.92	9.86±1.23	9.43±0.82	9.17±0.87	8.96±0.92	9.25±1.2
Gln	536.83±30.04	533.2±28.69	559.3±32.57	569.87±33.82	582.46±36.2	579.9±36.93	554.72±33.11	547.04±34.43	517.75±30.49
Glu	49.87±5.08	57.31±7.46	70.24±6.96	87.17±10.47	86.97±11.58	81.72±9.1	87.53±9.84	76.06±8.59	64.76±8.03
Gly	288.92±28.84	289.54±29.48	299.78±32.33	302.56±30.41	299.21±31.07	277.16±34.26	256.92±29.37	235.3±29.82	230.07±33.35
His	73.96±2.32	73.89±2.19	80.96±3.6	86.73±2.75	87.65±3.3	85.06±3.6	80.41±2.98	73.74±2.33	68.47±2.42
Hyp	8.49±0.88	9.13±0.91	9.85±0.98	9.98±0.99	9.9±0.92	9.19±0.99	8.53±1	7.71±0.87	6.8±0.65
Ile	75.33±3.65	119.89±8.62	190.86±12.49	226.84±13.85	224.98±11.32	218.16±8.92	208.44±10.88	173.61±11.08	118.68±11.27
Leu	132.73±4.68	216.33±13.77	358.27±24.4	430.49±27.09	439.93±21.23	443.08±18.02	447.55±21.93	388.87±22.61	282.2±23.27
Lys	162.58±10.15	213.25±13.45	305.29±21.95	354.78±27.7	371.22±25.35	356.06±25.26	347.52±23.57	304.79±24.78	228.84±19.75
Met	13.56±1.88	20.23±3.27	27.6±4.4	32.1±5.3	33.26±5.13	30.76±4.61	30.53±4.78	23.91±3.79	15.56±2.63
Orn	47.02±2.26	50.34±2.44	57.71±3.25	62.88±4.39	64.28±4.71	59.58±3.3	61.69±3.49	59.43±2.83	55.11±3.99
PEtN	2.46±0.24	2.22±0.18	2.8±0.2	2.77±0.17	2.66±0.2	2.45±0.17	2.24±0.18	1.98±0.14	2.13±0.19
Phe	58.9±2.07	70.05±2.4	85.93±3.24	91.65±3.67	91.23±2.76	87.64±2.43	83.76±3.13	72.76±3.69	58.84±4.53
Pro	179.29±11.13	202.12±11.09	242.01±12.35	261.82±13.57	261.05±12.88	260±12.38	256.87±14.12	234.55±14.22	204±11.55
Ser	101.99±5.83	111.22±5.55	131.9±6.07	142.89±6.3	147.77±11.59	124.59±6.4	116.2±4.98	97.89±5	92.47±11.75
Tau	63.2±4.69	62.65±4.54	62.29±4.29	63.7±3.94	61.78±3.95	56.38±3.91	54.13±4.29	50.34±3.34	50.97±3.64
Thr	135.24±7.14	156.32±9.08	202.85±13.01	230.73±15.84	228.85±14.17	224±15.55	218.07±15.83	195.81±11.36	170.03±10.27
Trp	40.71±1.71	49.53±2.2	67.45±3.43	79.82±4.01	84.98±3.44	90.82±3.09	94.32±3.24	88.57±2.78	72.37±3.16
Tyr	52.29±1.62	61.01±2.19	79.61±3.09	89.88±4.26	91.35±3.18	93.07±4.02	96.18±5.39	89.01±5.28	75.25±5.95
Val	234.54±11.38	278.75±15.34	352.27±19.33	401.57±23.61	408.1±20.5	408.07±20.58	411.12±21.57	376.94±15.02	308.7±13.34

Table 26: Prediabetic group: Postprandial amino acids after intake of CP drink

AA (µM)	0 min	15 min	30 min	45 min	60 min	90 min	120 min	180 min	240 min
Aad	1.08±0.09	1.04±0.09	1.22±0.07	1.74±0.08	2.69±0.16	3.4±0.23	4.1±0.26	3.94±0.27	2.96±0.24
Abu	26.68±2.02	27.89±2.41	30.23±2.3	32.01±2.51	31.98±2.31	32.19±2.46	30.43±2.11	27.54±1.73	26.83±1.54
Ala	401.54±20.31	409.46±20.46	478.98±26.54	535.01±30.12	574.39±30.22	588.76±28.4	559.34±28.36	474.69±27.99	455.09±25.74
Arg	93.72±5.2	107.04±5.53	140.61±5.8	146.24±6.17	150.33±6.85	143.17±5.79	130.23±6.37	110.13±5.66	96.7±5.28
Asn	50.93±2.23	57.7±2.27	77.56±3.07	88.84±3.27	90.2±3.55	83.3±3.26	71.8±3.52	55.65±2.41	50.36±2.59
Asp	2.62±0.25	2.63±0.21	3.6±0.23	5.27±0.4	7±1.05	5.33±0.68	4.26±0.38	3.41±0.44	2.71±0.56
Cit	33.09±1.85	32.17±2.84	35.13±5.65	36±4.2	37.07±3.52	40.5±3.06	43.36±2.87	46.81±3.34	41.37±1.97
Cys	261.78±14.82	256.95±13.83	260.8±14.86	273.73±12.85	262.73±13.92	265.13±13	258.6±15.42	231.88±10.56	227.17±12.69
EtN	9.68±0.96	9.03±0.87	9.04±0.87	9.37±1.03	9.88±0.96	10.04±0.94	10.12±0.99	9.1±0.82	9.85±1.03
Gln	556.89±34.29	548.54±28.13	585.94±31.42	614.06±37.3	627.39±36.47	611.71±37.4	587.46±37.6	532.47±32.32	526.63±29.93
Glu	54.55±6.79	58.38±6.74	64.98±7	81.45±7.02	88.7±9.22	87.35±10.7	78.39±8.83	70.15±6.74	49.83±6.88
Gly	263.68±13.38	266.16±13.29	282.87±15.51	292.1±14.36	286.86±14.26	266.25±15.92	245.93±13.87	220.12±9.59	216.98±8.6
His	76.81±3.22	76.95±2.75	90.75±3.33	100.79±3.65	101.47±3.47	90.34±3.53	85.43±3.86	73.12±3.43	71.22±3.49
Hyp	10.48±1.6	10.56±1.46	11.57±1.6	12.15±1.76	12.14±1.73	11.52±1.71	10.28±1.41	8.59±1.19	8.32±1.25
Ile	78.48±5.3	104.79±8.53	176.69±17.09	207.69±18.59	219.48±19.88	214.87±17.4	195.41±15.58	140.23±9.54	107.29±9.01
Leu	140.36±6.64	184.69±12.42	288.19±16.46	329.44±19.69	337.25±20.57	331.39±18.07	301.97±19.59	237.68±15.27	191.37±15.05
Lys	171.84±11.55	194.42±13.56	275.64±19.68	309.66±20.4	318.16±22.53	302.26±20.11	278.39±18.66	228.18±15.99	198.02±11.69
Met	13.34±1.85	18.05±3.13	31.41±4.49	34.33±5.09	36.6±6.03	36.94±6.6	33.13±4.89	23.32±3.52	22.33±3.48
Orn	52.24±3.38	56.93±4.24	70.71±6.74	75.93±6.23	81.47±7.84	78.66±5.95	76±4.96	71.71±4.25	61.54±3.49
PEtN	2.54±0.18	2.39±0.11	2.82±0.18	3.33±0.2	3.32±0.21	2.93±0.21	2.4±0.17	2.43±0.12	2.64±0.12
Phe	60.3±1.98	73.54±2.15	98.72±2.8	104.24±2.87	106.34±2.8	106.43±4.06	100.23±4.16	82.92±2.82	69.13±3.77
Pro	188.3±11.95	227.3±14.22	335.69±18.66	410.47±16.49	443.41±14.52	476.95±21.53	469.14±26.08	401.89±20.43	346.71±26.2
Ser	105.02±4.52	115.25±6.17	145.52±6.39	164.04±6.28	168.51±7.52	152.23±7.32	135.7±6.74	110.69±5.95	97.09±4.61
Tau	61.72±3.57	62.69±3.3	61.5±3.66	63.57±3.66	62.84±3.09	60.17±3.54	53.43±3.15	50.24±2.47	52.53±2.55
Thr	147.62±8.47	155.52±10.41	193.55±11.34	220.56±12.14	228.75±14.73	218.62±12.14	210.44±13.94	175.58±12.65	155.09±9.04
Trp	42.52±2.14	47.5±2	59.31±2.23	63.35±2.36	64.33±2.28	63.43±1.77	60.34±2.03	52.04±2.14	45.27±2.23
Tyr	55.55±2.4	67.53±3.57	103.2±6.39	116.19±7.2	122.55±7.19	128.25±7.21	126.93±6.7	108.64±5.57	96.57±6.59
Val	257.9±13.65	289.27±16.34	390.89±18.25	451.05±22.43	482.04±23.73	502±22.17	492.66±20.69	431.35±21.13	377.58±15.61

Table 27: Prediabetic group: Postprandial amino acids after intake of GMP drink

AA (µM)	0 min	15 min	30 min	45 min	60 min	90 min	120 min	180 min	240 min
Aad	1.04±0.08	0.97±0.06	1.12±0.08	1.37±0.09	1.68±0.15	2.55±0.17	3.2±0.28	3.53±0.24	2.54±0.26
Abu	24.86±1.25	27.51±1.53	32.89±1.78	37.25±2.17	39.88±2.54	42.55±2.32	47.75±2.67	52.02±3.46	52.42±3.67
Ala	414.62±22.45	468.16±21.81	601.92±33.9	688.58±38.05	742.64±39.59	783.38±49.74	728.9±47.43	658.27±38.62	565.21±28.11
Arg	89.04±4.15	94.35±3.52	102.62±4.42	103.7±3.93	104.02±4.78	100.96±5.24	93.97±4.92	83.67±3.78	75.87±3.07
Asn	50.8±1.93	68.46±4.39	105.02±6	124.88±5.16	126.07±5.07	122±8.13	107.07±6.87	84.45±6.55	63.28±3.36
Asp	2.58±0.28	3.17±0.28	4.46±0.46	6.55±0.73	8.26±0.92	8.01±0.9	9.65±1.57	5.31±1.01	3.47±0.53
Cit	31.28±2.94	31.91±3.55	30.37±3.86	31.02±4.72	31.95±4.57	33.51±3.51	37.61±3.53	36.68±2.69	35.51±2.51
Cys	256.16±13.39	255.11±10.25	263.31±12.46	274.18±13.25	271.26±12.56	273.19±12.56	260.15±11.34	256.53±13.26	247.6±10.12
EtN	9.73±0.95	9±1.03	9.02±1.01	8.76±0.92	9.08±0.83	9.17±0.82	9.22±0.71	8.92±0.91	8.87±0.85
Gln	563.01±19.95	561.35±15.82	598.76±16.04	626.3±17.87	628.51±23.6	627.05±24.38	605.25±24.11	584.21±18.04	561.83±16.41
Glu	54.3±7.44	61.93±7.24	71.27±9.15	97.24±10.69	108.43±13.31	104.12±13.46	111.72±12.97	82.05±8.3	54.89±6.74
Gly	277.1±23.72	281.94±21.33	302.17±24.39	308.5±27.7	305.95±25.94	291.73±26.44	288.09±25.11	260.54±23.95	253.08±26.52
His	71.71±3.1	73.56±2.85	77.41±2.51	75.81±3.27	76.17±3.57	69.95±3.1	66.95±2.95	64.29±2.9	67.11±2.71
Hyp	10.14±1.14	11.14±1.37	13.84±1.75	14.56±1.72	14.79±1.67	13.33±1.2	12.2±1.14	10.18±0.92	8.97±0.89
Ile	78.58±3.61	167.03±17.74	300.64±22.81	383.29±23.54	432.56±21.44	471.24±18.87	489.93±21.19	424.05±33.12	318.28±21.26
Leu	137.43±5.32	161.98±8.35	189.46±8.9	195.71±7.71	193.53±8.13	169.53±7.27	152.63±5.75	115.6±5.21	106.13±3.4
Lys	157.62±8.88	196.49±14.14	243.05±16.73	265.47±15.07	278.12±17.5	273.28±21.02	255.43±16.7	224.82±16.92	177.45±11.12
Met	14.96±2.18	23.7±4.07	30.93±4.91	34.42±5.73	33.84±5.3	34.2±5.38	33.7±5.37	24.15±3.78	18.86±3.06
Orn	46.55±3.11	52.14±3.66	60.01±3.77	60.68±3.62	62.06±3.96	54.65±2.89	54.91±3.48	52.58±2.98	50.78±2.98
PEtN	2.44±0.15	2.66±0.13	3.21±0.12	3.32±0.14	3.72±0.2	3.42±0.2	2.9±0.25	3.03±0.16	2.66±0.15
Phe	60.51±2.23	63.91±2.03	64.06±1.8	61.46±2.01	57.89±1.54	48.16±1.67	40.24±1.54	30.04±1.65	32.72±2.32
Pro	196.71±10.46	279.38±21.27	419.99±27.8	505.02±26.05	555.03±23.98	594.23±24.37	598.35±27.93	534.16±42.54	402.89±26.74
Ser	103.41±4.75	134.4±7.56	181.97±8.86	212.81±9.21	216.57±9.16	207.66±11.46	195.04±10.18	153.55±9.01	128.73±4.9
Tau	63.47±3.66	65.8±3.83	67.35±2.99	70.25±3.62	70.93±4.04	69.7±4.01	67.18±4.41	62.09±3.95	60.6±4.74
Thr	148.96±5.73	233.1±18.47	366.08±26.03	471.54±29.36	524.69±31.74	556.64±29.73	561.6±26.89	511.26±38.14	411.7±24.55
Trp	42.03±1.51	42.15±1.36	41.21±1.32	39.42±1.16	37.59±0.98	32.09±1.09	26.62±1.23	20.43±1.57	21.9±1.9
Tyr	53.34±2.24	53.66±2.23	53.76±2.46	51.13±2.1	48.63±2.03	42.8±1.93	36.32±1.42	27.49±1.42	26.91±1.51
Val	247.26±10.88	354.13±25.66	480.62±29.17	564.76±33.92	611.43±34.25	662.85±31.75	693.98±30.09	630.76±32.78	555.11±26.69

Table 28: Prediabetic group: Postprandial amino acids after intake of MD19 drink

AA (µM)	0 min	15 min	30 min	45 min	60 min	90 min	120 min	180 min	240 min
Aad	1.13±0.17	1.05±0.08	1.02±0.08	1.07±0.08	0.87±0.08	0.84±0.06	0.78±0.07	0.84±0.06	0.92±0.1
Abu	28.16±2.42	27.93±2.3	27.27±2.28	26.4±2.16	25.06±2.08	23.5±2.03	22.45±1.93	21.25±1.66	21.38±1.89
Ala	396.61±19.02	389.84±20.42	396.94±20.79	407.11±16.95	406.85±16.9	412.12±20.26	405.65±18.08	395.95±16.74	391.74±15.27
Arg	88.23±4.07	86.76±3.03	83.28±4.1	81.23±3.73	77.39±4.03	70.61±3.81	69.33±3.6	67.32±3.13	70.87±3.29
Asn	50.55±2.03	50.57±2.18	48.63±1.87	46.97±1.94	45.43±2.1	42.63±2.3	40.19±1.77	41.91±1.57	43.29±1.96
Asp	2.18±0.09	2.46±0.19	2.09±0.08	1.87±0.11	2.17±0.33	2.16±0.27	2.1±0.14	2.35±0.38	2.81±0.68
Cit	30.43±3.53	28.22±2.65	24.74±2.61	23.63±2.37	21.5±2.29	18.82±2.19	19.58±2.95	20.87±2.36	23.26±2.85
Cys	249.21±12.12	253.31±12.14	246.75±9.93	248.18±11.26	246.89±11.55	237.86±14.46	236.21±12.78	238.78±12.78	231.26±11.24
EtN	9.25±1.05	9.05±1.04	9.03±1.15	8.89±0.99	8.71±0.88	8.86±1	8.83±0.87	9.63±1.05	9.73±0.94
Gln	520.51±29.21	515.34±28.95	507.4±30.14	503.42±29.83	487.32±29.32	478.41±28.11	478.3±26.16	498.76±29.46	513.7±29.26
Glu	56.91±5.17	57.06±6.27	51.78±5.59	48.46±5.25	46.92±5.81	50.31±6.76	50.71±6.83	44.79±6.44	40.13±5.81
Gly	254.86±17.09	251.52±17.14	250.34±17.36	245.04±16.58	239.3±16.21	231.82±15.55	233.54±15.02	247.61±16.16	252.4±15.75
His	70.93±2.64	71.89±2.63	69.91±2.7	69.08±2.77	67.23±2.52	64.41±2.77	62.79±2.48	66.59±3.11	70.3±2.6
Hyp	9.36±1.44	9.3±1.36	8.92±1.36	8.86±1.41	8.45±1.23	7.95±1.2	7.62±1.17	7.3±1.05	7.43±1.2
Ile	82.07±4.75	81.48±5.13	76.75±4.25	71.02±3.93	66.34±3.7	54.76±2.96	48.4±2.62	45.79±2.21	51.28±2.26
Leu	138.78±6.44	139.27±6.9	132.87±6.23	125.57±5.76	115.12±5.62	98.06±4.97	89.2±4.63	86.52±3.93	96.45±4.04
Lys	160.27±9.57	162.22±9.31	156.45±9.92	153.45±8.82	146.44±8.64	141.04±9.34	140.49±8.8	143.18±8.5	149.99±9.02
Met	15.26±2.06	15.54±2.35	14.09±2	13.35±1.86	12.82±1.77	11.58±1.59	11.31±1.75	10.33±1.37	12.35±1.8
Orn	48.14±3.39	48.78±3.28	46.7±3.01	45.11±3.01	42.94±2.71	37.98±2.82	36.45±2.63	37.82±2.86	37.88±2.68
PEtN	2.31±0.1	2.42±0.13	2.27±0.11	2.25±0.11	2.1±0.1	2.42±0.42	2.12±0.12	2.12±0.12	2.34±0.13
Phe	60.09±1.59	58.6±1.67	57±1.63	54.29±1.71	52.49±1.57	47.66±1.78	45.67±1.67	46.54±1.77	51.1±1.72
Pro	180.14±7.18	178.86±8.11	173.55±8.05	167.48±7.89	164.89±7.26	153.92±7.28	150.04±6.19	146.35±6.46	145.47±6.39
Ser	98.04±4.78	98.84±4.96	95.94±4.62	90.63±4.28	89.54±4.07	82.98±4.19	80.73±3.78	83.11±4.97	89.52±5.41
Tau	63.18±3.43	65.63±4.03	63.32±3.76	62.57±3.54	61.1±3.18	61.69±7.22	58.2±3.47	55.42±3.29	56.02±2.9
Thr	140.09±9.22	137.04±6.96	134.91±7.24	129.91±7.27	124.53±6.33	116.77±7.03	110.87±6.86	110.4±7.42	116.06±7.35
Trp	41.83±1.69	41.86±1.71	40.92±1.64	40.56±1.49	39.64±1.45	37.2±1.57	35.94±1.62	35.86±1.57	36.57±1.49
Tyr	52.93±2.08	52.61±1.63	49.96±1.79	47.55±1.59	46.01±1.39	41±1.33	37.99±1.26	36.62±1.38	40.43±1.86
Val	249.37±13.08	253.31±14.91	247.95±13.6	236.8±13.61	226.84±14.49	205.8±12.69	195.12±12.23	189.06±10.07	199.21±12.31

Table 29: Control group: Amino acids AUC fcx30 min

	WPI	CP	GMP	MD19
Aad	33.42±1.38[a]	33.57±1.68[a]	31.06±1.43[a]	29.98±1.36[a]
Abu	32.53±1.03[a]	32.76±0.54[a]	35.68±0.57[b]	30.71±0.49[a]
Ala	35.70±1.00[a]	33.83±0.85[ab]	37.06±1.12[ab]	30.15± 1.04[b]
Arg	39.05±1.05[a]	41.33±1.29[a]	33.78±0.48[b]	29.02± 0.46[c]
Asn	40.69±1.28[a]	41.88±1.46[a]	48.62±2.03[b]	31.09± 1.21[c]
Asp	52.82±5.02[a]	39.26±2.24[a]	40.24±2.31[b]	29.11± 1.33[ab]
Cit	29.65±0.77[ab]	31.37±0.65[a]	29.19±0.68[ab]	27.60± 0.89[b]
Cys	35.84±1.30[a]	30.42±1.18[b]	31.65±0.59[abc]	29.66± 1.44[c]
Gln	32.01±0.34[a]	30.98±0.57[ab]	31.11±0.25[ab]	29.45± 0.65[b]
Glu	39.27±3.03[a]	40.44±3.42[a]	41.01±3.10[a]	32.07± 1.45[a]
Gly	32.20±0.53[ab]	32.31±0.50[ab]	31.97±0.50[bc]	30.19± 0.62[c]
His	32.57±0.47[ad]	33.82±1.03[ab]	31.08±0.40[d]	29.76± 0.60[cd]
Ile	56.33±3.89[a]	52.23±2.57[a]	78.23±4.15[b]	30.04± 1.73[c]
Leu	59.90±3.16[a]	51.20±2.42[b]	35.90±0.65[c]	28.46± 0.43[d]
Lys	48.11±1.77[a]	46.37±1.74[a]	40.66±1.09[b]	29.39± 0.73[c]
Met	48.78±1.80[a]	53.95±2.58[a]	48.28±2.74[a]	30.24± 1.14[b]
Orn	35.94±0.73[a]	37.28±1.02[a]	35.82±0.96[a]	29.95±1.23[b]
PETN	34.92±1.41[a]	34.20±1.50[a]	33.61±0.96[a]	27.37±0.99[b]
Phe	39.94±1.39[a]	42.08±1.34[a]	31.34±0.32[b]	28.25± 0.39[b]
Pro	36.81±1.12[a]	46.40±2.47[b]	48.42±1.99[b]	31.38± 1.86[a]
Ser	38.85±0.84[a]	41.07±1.22[ab]	44.90±1.50[b]	30.42± 0.77[c]
Tau	29.85±0.40[a]	30.82±0.38[a]	30.47±0.42[a]	29.61± 0.41[a]
Thr	40.55±1.56[a]	38.38±1.38[ab]	58.38±2.85[c]	33.76± 3.84[b]
Trp	43.33±2.11[a]	36.62±0.72[b]	30.18±0.53[c]	29.16± 0.32[cd]
Tyr	42.16±2.23[a]	47.87±2.32[a]	30.77±0.82[b]	28.32± 0.40[b]
Val	37.57±1.14[a]	39.96±1.28[a]	45.15±1.26[b]	29.94± 0.67[c]

Table 30: Control group: Amino acids AUC fcx30-90 min

	WPI	CP	GMP	MD19
Aad	173.25±13.49[b]	126.27±9.78[c]	81.02±5.25[a]	55.70±4.53[a]
Abu	73.29±2.96[a]	76.14±2.25[a]	97.32±2.29[b]	56.92±1.42[c]
Ala	96.47±4.26[ab]	93.90±3.54[ac]	110.76±5.05[d]	64.44±2.86[e]
Arg	92.37±3.06[ab]	101.57±3.48[ac]	72.42±2.43[d]	51.34±1.33[e]
Asn	104.65±4.08[ab]	116.48±4.52[ac]	145.33±6.22[d]	63.23±3.45[e]
Asp	187.45±19.75[b]	125.12±10.99[ac]	130.68±9.50[ad]	54.28±4.15[e]
Cit	69.64±2.86[ab]	72.40±2.83[ac]	60.64±2.05[d]	42.66±2.42[e]
Cys	90.86±1.99[b]	65.72±2.10[ac]	66.71±1.58[ad]	61.30±5.16[ae]
Gln	68.42±1.85[ab]	69.69±1.85[ac]	67.47±1.09[bc]	58.02±1.95[d]
Glu	101.96±8.80[ab]	96.97±10.45[ac]	120.97±11.55[bc]	59.05±3.80[d]
Gly	64.85±1.39[a]	70.84±1.59[a]	68.66±2.08[a]	58.64±1.43[b]
His	72.28±1.45[b]	81.30±2.71[c]	62.08±1.20[a]	58.44±1.87[a]
Ile	164.96±12.22[a]	160.71±8.69[a]	311.41±13.07[b]	48.35±4.28[c]
Leu	182.27±7.90[a]	143.90±7.51[b]	75.80±2.42[c]	46.29±0.99[d]
Lys	137.83±5.09[a]	125.96±4.76[a]	107.43±3.21[b]	54.46±1.92[c]
Met	131.81±4.94[ab]	158.51±11.35[a]	125.71±8.51[b]	57.34±8.08[c]
Orn	81.55±2.02[a]	94.52±3.26[b]	81.55±3.58[a]	54.52±2.60[c]
PETN	78.99±4.44[a]	88.37±6.16[a]	85.67±4.11[a]	47.38±2.73[b]
Phe	91.32±2.54[b]	105.66±4.07[c]	54.77±1.65[a]	50.41±1.17[a]
Pro	88.96±3.76[b]	144.85±9.23[a]	156.84±7.11[a]	62.03±4.66[c]
Ser	89.46±1.93[a]	106.54±4.36[b]	125.52±5.28[c]	57.67±1.95[d]
Tau	59.45±1.33[a]	62.29±1.58a[b]	64.39±1.37[b]	54.30±1.15[c]
Thr	107.18±5.32[a]	104.77±3.76[a]	214.73±9.69[b]	66.66±8.97[c]
Trp	122.11±5.26[b]	85.08±2.12[c]	51.89±1.84[a]	53.42±0.96[a]
Tyr	108.81±5.16[b]	137.21±7.59[c]	54.01±2.70[a]	49.39±1.33[a]
Val	96.16±4.61[a]	113.13±4.48[b]	136.49±4.26[c]	54.90±2.11[d]

Table 31: Control group: Amino acids AUC fcx90-240 min

	WPI	CP	GMP	MD19
Aad	716.59±56.27^{b}	390.76±26.32^{c}	230.44±18.70^{a}	138.11±10.51^{a}
Abu	147.99±6.49^{a}	159.90±5.46^{a}	249.21±6.95^{b}	125.27±2.49^{c}
Ala	214.17±7.41^{ab}	195.93±8.69^{a}	232.87±10.25^{b}	154.00±6.75^{c}
Arg	180.30±5.58^{a}	192.47±4.42^{a}	150.82±4.86^{b}	125.84±3.03^{c}
Asn	199.76±4.26^{a}	212.59±4.73^{a}	248.68±7.49^{b}	145.23±4.93^{c}
Asp	334.45±31.52^{b}	216.86±20.06^{a}	234.81±21.06^{a}	138.12±13.21^{c}
Cit	205.81±8.84^{a}	224.39±8.91^{a}	179.25±4.53^{b}	111.60±2.18^{c}
Cys	197.39±5.35^{b}	150.24±8.49^{a}	139.95±3.59^{a}	146.95±10.53^{a}
Gln	159.58±4.28^{a}	161.59±3.58^{a}	156.09±2.19^{a}	149.63±3.83^{a}
Glu	211.63±15.20^{a}	181.82±16.10^{ab}	222.45±25.48^{a}	124.10±10.14^{b}
Gly	135.25±3.36^{a}	146.97±3.09^{a}	148.16±3.90^{a}	146.99±3.81^{a}
His	156.80±3.14^{a}	167.99±3.79^{a}	141.69±2.61^{b}	145.72±4.01^{b}
Ile	285.16±16.44^{a}	276.80±9.91^{a}	707.87±15.03^{b}	98.09±3.40^{c}
Leu	357.69±10.94^{a}	263.34±8.14^{b}	134.50±2.27^{c}	104.49±2.64^{d}
Lys	271.66±6.81^{a}	234.39±6.40^{b}	209.54±4.92^{c}	137.76±3.26^{d}
Met	275.52±36.55^{a}	278.85±24.02^{a}	221.01±16.09^{ab}	130.25±21.17^{b}
Orn	179.46±4.20^{a}	213.90±6.58^{b}	171.23±7.28^{a}	122.68±2.04^{c}
PETN	158.40±7.03^{a}	181.70±9.04^{b}	174.30±6.13^{ab}	125.91±5.24^{c}
Phe	171.98±5.74^{a}	204.83±4.66^{b}	92.89±3.19^{c}	119.94±3.43^{d}
Pro	193.00±6.35^{b}	312.56±17.21^{a}	327.67±10.87^{a}	138.36±5.07^{c}
Ser	166.17±4.49^{a}	196.60±4.56^{b}	224.71±6.81^{c}	130.38±2.46^{d}
Tau	134.98±3.24^{a}	143.16±3.76^{ab}	147.75±2.34^{b}	135.52±2.75^{a}
Thr	221.54±8.72^{a}	215.75±9.58^{a}	465.43±12.87^{b}	134.50±3.49^{c}
Trp	278.67±11.80^{a}	174.24±4.15^{b}	91.72±3.05^{c}	124.23±2.75^{d}
Tyr	230.70±9.33^{b}	292.87±10.75^{c}	90.03±3.63^{a}	111.76±3.63^{a}
Val	206.07±8.75^{a}	256.02±5.56^{b}	336.12±7.48^{c}	122.22±2.24^{d}

Table 32: Control group: Amino acids AUC fcx240min

	WPI	CP	GMP	MD19
Aad	**923.26±69.3^{a}**	550.59±36.53^{b}	342.52±23.41^{c}	223.79±15.69^{c}
Abu	253.80±10.1^{a}	268.80±6.44^{a}	**382.21±8.59^{b}**	212.90±3.87^{c}
Ala	346.34±11.7^{ab}	323.66±11.19^{a}	380.68±15.11^{b}	248.58±10.12^{c}
Arg	311.72±8.32^{a}	335.38±6.65^{a}	257.02±7.43^{b}	206.20±4.44^{c}
Asn	345.10±8.09^{a}	370.95±5.46^{a}	**442.64±13.58^{b}**	239.55±9.36^{c}
Asp	**574.72±52.1^{a}**	381.24±30.20^{b}	405.73±30.18^{b}	221.51±17.74^{c}
Cit	305.10±12.1^{a}	328.15±11.89^{a}	269.08±6.84^{b}	181.86±4.67^{c}
Cys	**324.09±7.54^{a}**	246.38±7.40^{b}	238.31±5.07^{b}	237.91±16.72^{b}
Gln	260.01±6.14^{a}	262.26±4.52^{ab}	254.66±3.08^{ac}	237.11±6.30^{bc}
Glu	352.86±24.7^{a}	319.24±27.21^{ab}	384.43±39.11^{a}	215.23±13.16^{b}
Gly	232.31±4.74^{a}	250.12±4.46^{a}	248.79±6.18^{a}	235.83±5.26^{a}
His	261.65±4.30^{a}	283.11±4.72^{b}	234.85±4.00^{c}	233.93±6.11^{c}
Ile	506.45±29.8^{a}	489.74±16.72^{a}	**1097.51±9.59^{b}**	176.48±7.80^{c}
Leu	**599.86±16.5^{a}**	458.44±13.77^{b}	246.20±4.74^{c}	179.24±3.48^{d}
Lys	457.60±9.72^{a}	406.72±8.86^{b}	357.63±7.86^{c}	221.61±5.66^{d}
Met	456.11±39.1^{a}	491.32±35.17^{a}	395.00±25.59^{a}	217.82±29.81^{b}
Orn	296.95± 5.97^{a}	345.70± 9.33^{b}	288.59 ±11.62^{a}	207.15± 4.71^{c}
PETN	272.31±11.3^{a}	304.27±15.61^{a}	293.58±9.99^{a}	200.66±8.33^{b}
Phe	303.24±8.44^{b}	352.56±6.95^{c}	179.00±4.68^{a}	198.60±4.69^{a}
Pro	318.77±10.6^{a}	**503.81±24.47^{b}**	**532.93±17.65^{b}**	231±10.75^{c}
Ser	294.49±5.98^{a}	344.21±7.28^{b}	395.13±11.72^{c}	218.48±4.33^{d}
Tau	224.28±4.79^{a}	236.27±5.36^{a}	**242.61±3.78^{b}**	219.43±3.85^{a}
Thr	369.27±14.4^{a}	358.90±9.38^{a}	**738.54±20.81^{b}**	234.91±15.30^{c}
Trp	444.11±18.0^{a}	295.94±5.29^{b}	173.79±4.82^{c}	206.81±3.50^{c}
Tyr	381.67±16.0^{a}	477.94±18.36^{b}	174.81±6.85^{c}	189.47±5.20^{c}
Val	339.80±13.8^{a}	409.10±9.17^{b}	**517.77±11.06^{c}**	207.05±4.55^{d}

Table 33: Prediabetic group: Amio acids AUC fcx0-45 min

	WPI	CP	GMP	MD19
Aad	58.20±4.02^{a}	54.57±3.82^{ab}	48.85±1.83^{ab}	45.30±2.32^{b}
Abu	50.05±0.73^{a}	49.29±4.95^{a}	54.47±0.99^{b}	44.07±0.46^{c}
Ala	49.68±0.93^{a}	50.73±5.95^{a}	58.04±2.00^{b}	45.07±1.02^{c}
Arg	57.95±2.24^{a}	59.57±4.61^{a}	49.72±0.83^{b}	43.48±0.60^{c}
Asn	59.78±1.76^{a}	60.97±3.86^{a}	75.90±4.28^{b}	44.00±0.69^{c}
Asp	80.13±8.92^{a}	62.09±21.90^{ab}	74.80±6.19^{a}	45.39±1.43^{b}
Cit	45.59±1.44^{a}	45.40±6.64^{a}	44.23±1.26^{a}	40.02±0.67^{a}
Cys	51.14±1.44^{a}	45.36±3.25b^{c}	46.85±0.81^{c}	45.33±0.79^{c}
EtN	43.65±0.72^{a}	43.23±3.89^{a}	41.96±1.26^{a}	43.87±0.82^{a}
Gln	45.85±0.55^{ab}	46.74±2.65^{a}	46.87±0.63^{a}	44.23±0.66^{b}
Glu	59.17±2.50^{a}	56.47±14.30^{a}	62.33±5.27^{a}	42.96±2.03^{b}
Gly	45.99±0.70^{a}	47.09±1.94^{a}	47.48±0.63^{a}	44.29±0.58^{b}
His	47.86±1.04^{ab}	50.34±2.62^{a}	47.38±0.96^{ab}	44.84±0.48^{b}
Hyp	50.55±1.27^{ab}	48.84±2.60^{a}	54.11±1.58^{b}	44.00±0.51^{c}
Ile	92.60±4.37^{a}	81.76±18.00^{a}	128.06±10.94^{b}	42.94±0.45^{c}
Leu	97.20±5.08^{a}	76.22±15.14^{b}	55.55±1.48^{c}	43.70±0.41^{c}
Lys	72.52±3.87^{a}	62.18±5.27b^{c}	61.43±3.08^{c}	44.56±0.58^{d}
Met	77.50±5.42^{a}	82.88±9.49^{a}	79.78±6.27^{a}	43.19±1.21^{b}
Orn	52.27±1.66^{a}	55.00±6.62^{a}	52.79±1.58^{a}	44.56±0.78^{b}
PEtN	50.46±3.74^{a}	50.33±5.57^{a}	54.26±3.30^{a}	45.87±1.56^{a}
Phe	59.35±3.74^{a}	63.87±5.78^{b}	46.78±0.60^{c}	43.13±0.32^{c}
Pro	56.24±1.81^{a}	69.78±18.38^{b}	79.78±5.11^{c}	43.73±0.32^{d}
Ser	54.67±1.811^{a}	56.75±4.53^{a}	68.35±3.13^{b}	44.36±0.72^{c}
Tau	45.00±0.68^{a}	45.56±2.57^{a}	47.60±1.00^{a}	45.48±0.85^{a}
Thr	60.38±2.11^{a}	54.33±4.51^{ab}	89.74±6.26^{c}	43.98±0.76^{b}
Trp	65.90±2.46^{a}	57.00±1.29^{b}	44.14±0.44^{c}	44.52±0.39^{c}
Tyr	61.20±2.15^{a}	69.47±12.80^{b}	44.80±0.48^{c}	43.42±0.34^{c}
Val	60.83±1.75^{a}	60.60±9.47^{a}	72.97±3.54^{b}	44.68±0.32^{c}

Table 34: Prediabetic group: Amino acids AUC fcx45-120 min

	WPI	CP	GMP	MD19
Aad	834.51±64.28^{a}	557.93±80.65^{b}	403.10±31.01^{cb}	156.96±9.50^{d}
Abu	210.92±5.20^{a}	206.02±4.63^{a}	291.33±12.13^{b}	155.18±1.99^{c}
Ala	225.06±7.71^{a}	236.55±6.11^{a}	290.19±15.05^{b}	184.30±5.57^{c}
Arg	243.05±8.38^{a}	253.15±5.66^{a}	193.25±3.80^{b}	152.51±3.05^{c}
Asn	255.20±8.83^{a}	261.42±7.00^{a}	352.68±19.45^{b}	157.23±2.81^{c}
Asp	529.00±49.28^{a}	326.31±29.51^{b}	467.58±44.22^{a}	181.44±9.45^{c}
Cit	230.65±7.30^{a}	216.49±8.03^{ab}	192.49±7.74^{b}	130.38±2.72^{c}
Cys	231.42±8.07^{a}	178.64±4.68^{b}	188.65±4.53^{b}	175.54±4.38^{b}
EtN	178.42±3.27^{a}	181.63±6.10^{a}	171.31±6.72^{a}	177.00±3.90^{a}
Gln	186.51±3.59^{a}	189.13±3.111^{a}	192.99±3.75^{b}	170.59±3.61^{c}
Glu	292.02±16.79^{a}	273.93±18.95^{a}	334.05±28.11^{a}	158.05±10.84^{b}
Gly	169.39±3.86^{ac}	177.52±2.91^{abc}	186.17±4.33^{b}	170.55±2.62^{c}
His	197.71±5.81^{a}	204.39±2.92^{a}	177.70±3.86^{b}	168.66±1.95^{b}
Hyp	191.62±5.67^{a}	188.08±3.45^{a}	220.14±7.55^{b}	157.56±1.94^{c}
Ile	469.84±16.88^{a}	419.26±19.32^{a}	868.18±69.55^{b}	131.56±2.25^{c}
Leu	542.80±22.14^{a}	369.78±18.81^{b}	205.00±6.7^{c}	138.74±3.86^{d}
Lys	360.41±16.68^{a}	283.78±10.82^{bc}	273.90±14.03^{c}	165.08±2.83^{d}
Met	363.24±26.90^{a}	404.25±14.54^{a}	348.70±20.00^{a}	144.17±4.66^{b}
Orn	229.40±8.76^{ab}	254.33±7.51^{a}	211.62±8.34^{b}	153.33±2.74^{c}
PEtN	190.75±16.12^{ab}	201.02±12.53^{ab}	227.73±14.46^{a}	175.32±7.64^{b}
Phe	253.37±7.16^{a}	287.13±7.67^{b}	145.17±2.05^{c}	150.58±1.74^{c}
Pro	249.90±7.46^{a}	401.12±18.86^{bc}	460.33±34.03^{c}	158.78±1.95^{d}
Ser	217.87±7.98^{a}	239.72±7.94^{a}	313.39±18.54^{b}	160.29±3.21^{c}
Tau	164.62±3.33^{a}	169.57±2.71^{a}	189.38±3.87^{b}	171.11±3.40^{c}
Thr	277.46±10.46^{a}	245.12±6.06^{a}	545.66±34.61^{b}	156.07±3.76^{c}
Trp	166.60±7.98^{a}	113.40±3.72^{b}	60.00±1.18^{c}	68.40±1.07^{c}
Tyr	302.76±12.34^{a}	368.04±14.45^{b}	142.90±1.76^{c}	146.47±1.75^{c}
Val	294.37±8.53^{a}	314.19±9.85^{a}	409.47±22.77^{b}	154.70±2.02^{c}

Table 35: Prediabetic group: Amino acids AUC fcx120-240 min

	WPI	CP	GMP	MD19
Aad	881.74±85.45	471.77±68.95	361.50±37.78	99.36±7.97
Abu	137.07±5.30[a]	129.73±4.95[a]	232.82±15.82[b]	92.45±1.16[c]
Ala	142.10±6.90[ac]	148.08±5.95[a]	186.41±12.01[b]	121.69±4.54[c]
Arg	141.44±4.68[a]	144.96±4.61[a]	113.52±2.58[b]	93.79±2.26[c]
Asn	143.70±5.71[a]	138.87±3.86[a]	190.11±13.63[b]	99.60±1.81[c]
Asp	322.76±33.41[a]	174.01±21.90[b]	252.83±30.81[ab]	135.69±17.[30c]
Cit	181.20±6.41[a]	162.72±6.64[ad]	138.45±8.24[bd]	84.10±2.75[c]
Cys	147.61±4.92[a]	110.15±3.25[bc]	121.97±4.07[c]	114.25±3.16[c]
EtN	116.34±3.01[a]	119.88±3.89[a]	113.74±5.43[a]	124.75±3.62[a]
Gln	120.43±3.14[ab]	117.42±2.65[ab]	125.12±3.45[a]	114.82±2.58[b]
Glu	191.52±12.62[a]	164.31±14.29[a]	199.09±19.95[a]	96.80±9.38[c]
Gly	97.65±2.80[a]	103.43±1.95[a]	115.09±3.02[b]	115.83±2.01[b]
His	120.89±3.27[a]	118.78±2.62[ab]	110.59±3.29[b]	112.58±1.79[ab]
Hyp	109.26±3.32[a]	105.35±2.60[ad]	122.73±4.62[b]	95.77±1.71[cd]
Ile	276.89±18.47[a]	233.95±18.00[a]	593.44±64.48[b]	70.84±1.85[c]
Leu	349.66±22.85[a]	212.79±15.14[b]	103.72±4.60[c]	78.48±3.07[c]
Lys	219.05±11.27[a]	165.38±5.27[b]	161.98±9.25[cb]	108.30±2.60[d]
Met	197.65±13.39[a]	224.86±9.49[a]	188.14±11.42[a]	88.23±4.53[b]
Orn	152.72±7.25[ad]	164.37±6.62[a]	133.14±5.75[bd]	93.87±2.92[c]
PEtN	109.53±8.52[a]	121.30±5.57[ab]	140.06±8.90[b]	114.48±4.52[ab]
Phe	147.39±5.69[a]	168.27±5.78[b]	67.76±2.77[c]	94.62±1.61[d]
Pro	157.66±6.21[a]	267.25±18.38[bc]	303.24±29.56[c]	98.03±1.75[da]
Ser	120.64±4.73[a]	130.76±4.53[a]	177.86±12.07[c]	103.50±3.59[da]
Tau	98.37±2.17[a]	101.49±2.57[ac]	117.04±2.06[b]	106.98±2.08[c]
Thr	174.42±7.87[a]	145.99±4.51[ac]	369.79±28.49[b]	96.34±2.81[c]
Trp	259.40±12.42[a]	150.80±5.65[b]	66.00±3.81[c]	103.70±2.09[d]
Tyr	202.12±12.30[a]	241.56±12.80[b]	67.84±2.05[c]	86.27±1.87[c]
Val	192.28±8.70[a]	206.32±9.47[a]	285.84±21.20[b]	92.91±1.73[c]

Table 36: Prediabetic group: Amino acids AUC fcx240 min

	WPI	CP	GMP	MD19
Aad	**1280.16±103.45[a]**	776.31±109.30[bc]	574.81±48.17[c]	208.03±13.18[d]
Abu	277.79±7.69[a]	269.17±7.16[a]	**409.91±20.77[b]**	200.85±2.32[c]
Ala	292.49±10.33[a]	306.53±8.58[a]	**377.72±20.07[b]**	244.63±7.70[c]
Arg	308.04±10.01[a]	320.48±8.04[a]	247.45±4.67[b]	199.72±4.13[c]
Asn	321.14±10.63[a]	324.53±8.16[a]	**435.81±24.08[b]**	207.96±3.51[c]
Asp	**671.09±53.59[a]**	404.59±39.78[b]	564.47±52.54[a]	254.10±18.25[b]
Cit	322.47±9.89[a]	297.45±10.75[ab]	261.20±11.54[b]	174.58±4.14[c]
Cys	**301.27±9.72[a]**	231.95±6.19[b]	248.89±6.64[b]	232.42±5.85[b]
EtN	236.33±4.60[a]	241.07±7.84[a]	227.69±9.36[a]	240.99±5.69[a]
Gln	245.74±4.97[a]	246.33±4.34[a]	254.66±5.25[a]	228.94±4.78[b]
Glu	380.53±19.73[a]	347.54±24.71[a]	417.65±36.15[a]	203.90±15.18[b]
Gly	216.55±5.08[a]	227.70±3.58[ab]	242.07±5.36[ab]	229.61±3.59[b]
His	255.63±6.93[a]	261.12±4.24[a]	233.41±5.54[b]	226.58±2.79[bc]
Hyp	243.37±6.85[a]	237.92±4.68[a]	276.70±9.37[b]	205.22±2.68[c]
Ile	591.18±23.47[a]	521.04±28.97[b]	**1133.23±96.76[c]**	167.63±3.11[c]
Leu	**699.68±30.36[a]**	465.68±26.39[b]	252.38±7.74[c]	178.78±5.01[d]
Lys	458.63±19.94[a]	360.34±11.08[b]	348.10±17.47[b]	220.19±4.24[c]
Met	447.12±30.62[a]	505.28±16.92[a]	429.73±24.10[a]	189.98±6.75[b]
Orn	303.96±11.97[ab]	332.71±10.53[a]	276.96±11.20[b]	200.89±4.51[c]
PEtN	244.05±19.33[a]	263.30±14.33[a]	296.10±18[a]	234.05±10.01[a]
Phe	320.44±8.61[a]	363.63±9.80[b]	177.62±3.24[c]	199.28±2.47[c]
Pro	324.18±9.67[a]	524.84±27.33[b]	596.79±46.44[b]	207.42±2.81[c]
Ser	274.51±9.29[a]	299.48±9.48[b]	393.85±23.31[c]	213.42±5.18[d]
Tau	213.16±4.22[a]	220.29±3.90[a]	246.09±4.54[b]	224.10±3.93[a]
Thr	359.66±13.59[a]	312.69±7.91[a]	**716.00±46.22[b]**	204.80±5.07[c]
Trp	491.90±20.04[a]	321.20±9.88[b]	170.20±4.55[c]	216.60±3.31[d]
Tyr	397.77±17.32[a]	480.91±20.73[b]	174.58±2.71[c]	190.27±2.64[c]
Val	384.39±12.01[a]	411.10±14.61[a]	**544.24±32.31[b]**	201.42±2.82[c]

Table 37: Control group: Amino acids AUC fcx90 min

	WPI	CP	GMP	MD19
Aad	**206.67±5.44**[a]	159.84±7.51[b]	112.08±3.61[c]	85.68±4.92[c]
Abu	105.82±1.67[a]	108.9±1.83[a]	**133±1.51**[b]	87.63±1.75[c]
Ala	132.17±5.05[a]	127.73±5.86[a]	147.82±4.57[b]	94.58±7.75[c]
Arg	131.41±2.27[a]	142.91±5.43[a]	106.2±2.68[b]	80.36±6.41[c]
Asn	145.34±44.35[a]	158.36±91.6[a]	193.96±84.89[b]	94.32±4.63[c]
Asp	240.27±1.72[a]	164.38±1.96[b]	170.93±1.83[bc]	83.39±2.53[d]
Cit	99.29±24.16[ab]	103.77±12.09[a]	89.83±5.25[b]	70.25±11.25[c]
Cys	**126.7±6.22**[a]	96.14±12.49[b]	98.36±6.8[b]	90.96±32.34[b]
Gln	100.43±5.16[a]	100.67±4.16[a]	98.57±3.6[a]	87.48±5.93[b]
Glu	141.23±6.66[a]	137.42±5.03[a]	161.98±12.77[a]	91.13±11.76[b]
Gly	97.06±11.42[a]	103.15±13.72[a]	100.63±5.03[a]	88.83±14.2[b]
His	104.86±1.82[a]	115.11±3.66[b]	93.16±2.27[c]	88.2±1.54[c]
Ile	221.29±3.88[a]	212.94±2.68[a]	**389.64±1.74**[b]	78.39±2.71[c]
Leu	**242.17±14.72**[a]	195.1±11.34[b]	111.69±5.71[c]	74.75±5.59[d]
Lys	185.94±4.74[a]	172.33±11.63[a]	148.09±6.47[b]	83.85±8.58[c]
Met	180.59±3.89[ab]	212.47±4.58[b]	173.99±1.76[a]	87.57±2.84[c]
Orn	117.49±2.41[a]	131.81±4.19[b]	117.36±3.76[ac]	84.47±4.46[d]
PEtN	113.91±3.07[a]	122.57±3.24[a]	119.28±6.35[a]	74.75±1.99[b]
Phe	130.46±6.35[a]	147.73±6.32[b]	86.11±2.61[c]	78.66±4.01[c]
Pro	125.77±5.58[a]	191.25±5.69[b]	**205.26±2.76**[bc]	93.41±5.19[d]
Ser	128.32±5.75[a]	147.61±13.64[b]	**170.43±9.03**[c]	88.09±10.9[d]
Tau	89.3±7.17[ab]	93.11±9.72[a]	**94.86±1.64**[b]	83.91±3.5[b]
Thr	147.73±15.66[a]	143.15±11[a]	**273.11±6.01**[b]	100.42±16.03[c]
Trp	166.99±10.39[a]	121.7±9.72[b]	**82.07±1.25**[c]	82.58±2.91[c]
Tyr	150.97±3.87[a]	185.08±5.3[b]	**84.78±1.43**[c]	77.71±1.92[c]
Val	133.73±7[a]	153.08±2.77[b]	**181.65±1.2**[c]	84.84±2.36[d]
Total	3850.89±11.44[a]	3830.83±9.47[a]	3858.35±17.38[a]	2299.64±1.76[b]

Table 38: Prediabetic group: Amino acids AUC fcx90 min

	WPI	CP	GMP	MD19
Aad	**207.31±21.97**[a]	172.07±23.38[ab]	131.79±17.95[bc]	79.19±6.95[c]
Abu	105.18±1.93[a]	103.84±1.85[a]	**127.92±5.44**[b]	84.26±1.05[c]
Ala	110.54±3.04[a]	115.14±2.00[a]	140.34±7.58[b]	92.63±1.69[c]
Arg	125.01±4.98[a]	131.25±3.67[a]	102.65±11.38[b]	82.54±1.43[c]
Asn	133.38±4.75[a]	139.42±3.71[a]	184.88±11.38[b]	84.32±1.69[c]
Asp	249.91±30.51[a]	172.56±15.41[b]	220.80±26.45[ab]	90.20±4.84[c]
Cit	100.10±3.34[a]	96.71±4.51[a]	90.74±3.24[a]	71.79±1.42[b]
Cys	**112.30±3.98**[a]	91.51±2.28[b]	96.68±2.91[b]	90.51±2.22[b]
Gln	93.91±1.55[ab]	96.77±1.18[a]	97.77±1.54[a]	86.87±1.62[b]
Glu	136.89±8.03[a]	134.35±8.97[a]	156.42±14.2[a]	81.21±4.41[b]
Gly	91.45±1.9[ab]	95.13±1.22[a]	97.14±2.62[a]	86.99±1.56[b]
His	100.90±2.72[ab]	107.90±1.68[b]	95.02±2.51[a]	87.71±1.18[c]
Ile	227.97±10.09[a]	207.12±9.29[a]	**372.62±27.6**[b]	77.89±1.04[c]
Leu	**248.00±11.84**[a]	184.37±9.13[b]	115.61±3.38[c]	79.97±1.27[d]
Lys	174.09±9.51[a]	144.02±5.65[b]	139.98±8.62[bc]	86.13±1.36[d]
Met	183.86±14.85[a]	203.94±8.30[a]	181.44±13.97[a]	80.19±2.06[b]
Orn	112.31±4.08[a]	123.60±4.17[a]	110.71±4.79[a]	84.10±1.68[b]
PETN	102.93±8.25[ab]	109.44±7.55[ab]	119.20±7.52[a]	90.97±4.65[b]
Phe	128.95±4.08[a]	143.69±7.55[b]	88.11±1.30[c]	81.78±0.77[c]
Pro	122.62±3.08[a]	180.12±6.41[b]	**209.24±14.77**[b]	84.59±0.91[c]
Ser	117.81±4.98[a]	127.13±3.67[a]	**163.01±11.38**[b]	85.47±1.63[c]
Tau	88.48±1.82[a]	91.13±1.37[a]	**98.52±3.09**[b]	89.93±2.09[a]
Thr	136.31±5.34[a]	122.90±2.80[a]	**247.76±20.84**[b]	84.28±1.76[c]
Trp	162.78±7.01[a]	125.85±3.49[b]	**83.31±1.17**[c]	87.25±1.26[c]
Tyr	140.97±5.47[a]	169.91±6.56[b]	**84.95±1.07**[c]	81.90±0.98[c]
Val	139.39±4.16[a]	145.97±4.03[a]	**184.32±10.32**[b]	84.85±0.91[c]
Total	3729.49±12.37[a]	3604.05±9.31[a]	3799.60±16.98[a]	2289.79±1.26[b]

Table 39: Summary of AUC of BCAAs, AAAs, EAAs, NEAAs and TAAs at different time periods

Control group: Amino acids AUC fcx0-30 min

	WPI	CP	GMP	MD19
BCAAs	153.80±7.05^{a}	143.38±6.18^{a}	159.28±5.76^{a}	88.44±2.44^{b}
AAAs	125.43±5.63^{a}	126.57±4.30^{a}	92.30±1.62^{b}	85.73±0.84^{b}
EAAs	374.50±13.07^{a}	360.78±13.12^{a}	368.13±11.29^{a}	239.23±6.74^{b}
NEAAs	457.97±11.01^{a}	459.62±12.51^{a}	450.62±9.07^{a}	360.63±8.58^{b}
TAAs	**832.47±22.21^{a}**	**820.40±24.91^{a}**	**818.75±18.80^{a}**	**599.86±15.19^{b}**

Control group: Amino acids AUC fcx30-90 min

	WPI	CP	GMP	MD19
BCAAs	443.39±22.29^{a}	417.74±20.31^{a}	523.71±18.42^{b}	149.53±7.12^{c}
AAAs	322.24±12.25^{a}	327.94±13.38^{a}	160.66±6.02^{b}	153.23±2.92^{b}
EAAs	1033.64±35.77^{a}	997.72±42.60^{a}	1078.22±36.91^{a}	431.82±17.97^{b}
NEAAs	1166.55±33.25^{a}	1210.19±36.13^{a}	1181.46±36.30^{a}	697.85±26.63^{b}
TAAs	**2200.19±63.66^{a}**	**2207.91±76.74^{a}**	**2259.68±68.82^{a}**	**1129.67±43.88^{b}**

Control group: Amino acids AUC fcx90-240 min

	WPI	CP	GMP	MD19
BCAAs	848.92±27.37^{a}	796.15±22.62^{a}	1178.49±21.13^{b}	324.80±7.71^{c}
AAAs	681.36±24.48^{a}	671.93±17.14^{a}	274.65±9.04^{b}	355.93±8.69^{c}
EAAs	2068.29±64.70^{a}	1904.22±49.60^{b}	2259.09±39.88^{c}	971.48±23.43^{d}
NEAAs	2379.20±47.87^{a}	2428.49±64.29^{a}	2317.92±67.54^{a}	1657.08±41.18^{b}
TAAs	**4447.49±94.51^{a}**	**4332.70±98.37^{a}**	**4577±92.11^{a}**	**2628.56±57.90^{b}**

Control group: Amino acids AUC fcx240 min

	WPI	CP	GMP	MD19
BCAAs	1446.11±46.06^{a}	1357.28±39.72^{a}	1861.48±33.44^{b}	562.77±15.13^{c}
AAAs	1129.02±40.39^{a}	1126.45±29.17^{a}	527.60±15.93^{b}	594.89±12.13^{b}
EAAs	3476.43±85.59^{ab}	3262.72±81.00^{a}	3705.44±75.55^{b}	1642.53±41.57^{c}
NEAAs	4003.72±77.62^{a}	4098.30±72.05^{a}	3950±102.42^{a}	2715.56±74.47^{b}
TAAs	**7480.15±138.55^{a}**	**7361.02±133.96^{a}**	**7655.44±154.75^{a}**	**4358.09±111.52^{b}**

Prediabetic group: Summary of amino acids AUC fcx45 min

	WPI	CP	GMP	MD19
BCAAs	250.64±11.30^{a}	218.57±6.51^{b}	256.58±1.00^{a}	131.33±16.32^{c}
AAAs	186.42±6.51^{a}	190.34±5.31^{a}	135.73±1.40^{b}	131.07±0.94^{b}
EAAs	586.26±25.23^{a}	538.83±12.62^{a}	578.46±30.10^{a}	350.71±2.91^{b}
NEAAs	669.67±19.14^{a}	675.36±11.92^{a}	702.32±3.83^{a}	531.10±21.58^{b}
TAAs	**1255.93±42.87^{a}**	**1214.19±22.71^{a}**	**1280.78±49.29^{a}**	**881.81±13.92^{b}**

Prediabetic group: Summary of amino acids AUC fcx45-120 min

	WPI	CP	GMP	MD19
BCAAs	1307.00±47.22^{ab}	1103.24±36.80^{a}	1482.65±100.38^{b}	425.00±6.02^{c}
AAAs	722.78±26.76^{a}	768.54±24.44^{a}	348.06±4.38^{b}	365.434.02^{b}
EAAs	2728.12±102.32^{a}	2436.90±59.44^{a}	2856.06±150.20^{ab}	1109.29±15.51^{c}
NEAAs	3099.89±87.68^{a}	3109.92±68.38^{a}	3299.89±131.82^{a}	1984.43±24.28^{b}
TAAs	**5828.01±167.74^{a}**	**5546.82±113.80^{a}**	**6155.95±268.63^{a}**	**3160.76±36.34^{b}**

Prediabetic group: Summary of amino acids AUC fcx120-240 min

	WPI	CP	GMP	MD19
BCAAs	818.83±50.21^{ab}	653.06±40.32^{b}	983.00±92.32^{ac}	242.23±5.52^{d}
AAAs	608.88±29.27^{a}	560.63±22.98^{a}	201.64±8.44^{b}	284.58±5.03^{c}
EAAs	1816.72±85.56^{a}	1508.37±61.76^{b}	1836.71±124.2^{a}	733.41±13.60^{c}
NEAAs	1908.52±65.73^{a}	1859.57±59.86^{a}	1963.69±102.40^{a}	1292.85±30.41^{b}
TAAs	**3725.24±143.77^{a}**	**3367.93±114.62^{a}**	**3800.40±219.23**	**2026.26±40.53**

Prediabetic group: Summary of amino acids AUC fcx240 min

	WPI	CP	GMP	MD19
BCAAs	1675.25±65.94^{ab}	1397.82±60.34^{b}	1929.85±139.61^{a}	547.82±8.44^{c}
AAAs	1210.10±44.57^{a}	1165.71±38.02^{a}	522.35±9.85^{b}	606.13±7.53^{c}
EAAs	3752.99±132.71^{ab}	3260.93±93.83^{a}	3771.46±198.98^{b}	1578.64±22.39^{c}
NEAAs	3988.96±98.84^{a}	3976.02±94.46^{a}	4187.33±169.54^{a}	2638.97±41.22^{b}
TAAs	**7741.94±206.99^{a}**	**7236.95±172.31^{a}**	**7958.79±353.35^{a}**	**4217.62±58.93^{b}**

Table 40: Control group: Minimum and maximum fold changes and times of amino acids after intake of different test meals

	WPI				CP				GMP				MD19			
	Min	min	Max	min	Min	min	Max	min	Min	min	Max	min	Min	min	Max	min
Aad	1±0.00	0	5.29±0.50^{a}	120	1±0.00	0	2.97±0.21^{b}	120	1±0.00	0	1.77±0.14^{cd}	120	0.85±0.21	120	1.03±0.16^{d}	30
Abu	0.83±0.04	240	1.24±0.05^{a}	60	0.95±0.03	240	1.29±0.05^{a}	60	1±0.00	0	1.77±0.05^{b}	90	0.81±0.02	120	1.05±0.05^{c}	30
Ala	1±0.00	0	1.66±0.08^{a}	60	1±0.00	0	1.64±0.07^{a}	90	1±0.00	0	1.99±0.10^{b}	90	0.97±0.03	15	1.09±0.11^{c}	30
Arg	0.9±0.03	240	1.55±0.04^{a}	30	0.99±0.03	240	1.73±0.05^{b}	60	0.87±0.03	240	1.22±0.05^{c}	60	0.8±0.02	120	1±0.00^{d}	0
Asn	0.99±0.03	240	1.16±0.06^{a}	30	1±0.00	0	1.98±0.10^{a}	60	1±0.00	0	2.51±0.12^{b}	90	0.95±0.03	180	1.16±0.13^{c}	30
Asp	1±0.00	0	3.56±0.44^{a}	120	1±0.00	0	2.95±0.64^{a}	90	0.96±0.16	240	2.52±0.30^{ab}	90	0.87±0.17	60	1±0.00^{b}	0
Cit	0.98±0.03	30	1.46±0.10^{a}	120	1±0.00	0	1.6±0.06^{ab}	120	0.95±0.03	30	1.29±0.04^{ac}	120	0.64±0.03	90	1±0.00^{d}	0
Cys	1±0.00	0	1.55±0.05^{a}	90	0.95±0.07	240	1.12±0.08^{b}	90	0.87±0.04	240	1.14±0.03^{b}	30	0.93±0.05	15	1.09±0.06^{b}	30
EtN	0.89±0.09	180	1±0.00^{a}	0	0.53±0.54	180	1.16±0.14^{a}	30	0.73±0.17	60	1.84±0.92	30^{a}	0.83±0.06	15	1.02±0.11^{a}	240
Gln	1±0.00	0	1.15±0.03^{a}	60	1±0.00	0	1.18±0.04^{a}	60	1±0.00	180	1.13±0.03^{a}	60	0.93±0.02	120	1.06±0.03^{a}	240
Glu	0.93±0.11	240	1.82±0.20^{a}	60	0.81±0.10	240	1.69±0.23^{ab}	30	0.88±0.10	240	2.15±0.24^{ab}	60	0.72±0.09	240	1.15±0.23^{b}	30
Gly	0.82±0.03	240	1.14±0.03^{a}	30	0.91±0.02	240	1.2±0.02^{a}	30	0.92±0.03	240	1.15±0.04^{a}	90	0.96±0.02	60	1.01±0.04^{b}	15
His	0.96±0.02	240	1.22±0.20^{a}	60	1±0.00	240	1.69±0.06^{a}	30	0.93±0.02	180	1.07±0.02^{b}	30	0.93±0.03	120	1.02±0.03^{b}	240
Ile	0.98±	240	2.78±0.22^{a}	90	1±0.00	0	2.8±0.18^{a}	60	1±0.00	0	6.13±0.28^{b}	90	0.57±0.08	120	1.11±0.14^{c}	30
Leu	1±0.07	0	3.17±0.14^{a}	120	1±0.00	0	2.47±0.11^{b}	60	0.79±0.02	180	1.36±0.04^{c}	30	0.61±0.08	120	1±0.00^{d}	0
Lys	1±0.00	0	2.35±0.08^{a}	90	1±0.00	0	2.15±0.09^{a}	60	1±0.00	0	1.87±0.06^{b}	90	0.88±0.02	90	1±0.00^{c}	15
Met	1±0.00	0	2.24±0.10^{a}	60	1±0.00	0	2.72±0.19^{b}	60	1±0.00	0	2.13±0.14^{a}	90	0.83±0.23	120	1.08±0.13^{c}	30
Orn	0.97±0.03	240	1.37±0.04^{a}	60	1±0.00	0	1.66±0.05^{a}	90	1±0.00	0	1.42±0.06^{b}	30	0.85±0.21	120	1.04±0.14^{c}	30
PEtN	0.97±0.05	180	1.47±0.09^{a}	60	1±0.00	0	1.59±0.13^{a}	60	1±0.00	0	1.47±0.08^{a}	60	0.77±0.04	90	1±0.00^{b}	0
Phe	0.84±0.03	240	1.55±0.06^{a}	30	1±0.00	0	1.79±0.09^{b}	60	0.56±0.03	180	1.07±0.01^{c}	15	0.75±0.03	120	1±0.00^{c}	0
Pro	1±0.00	0	1.5±0.07^{a}	60	1±0.00	0	2.63±0.16^{b}	90	1±0.00	0	2.92±0.15^{b}	90	0.9±0.04	180	1.11±0.24^{a}	30
Ser	0.85±0.03	240	1.08±0.05^{a}	30	1±0.00	0	1.79±0.09^{a}	60	1±0.00	0	2.13±0.10^{b}	90	0.85±0.02	120	1.08±0.09^{c}	30
Tau	0.87±0.02	180	1.02±0.03^{a}	30	0.92±0.02	180	1.05±0.03^{a}	60	0.92±0.03	240	1.09±0.03^{a}	60	0.89±0.03	120	1.01±0.02^{a}	15
Thr	1±0.00	0	1.82±0.10^{a}	60	1±0.00	0	1.82±0.08^{a}	90	1±0.00	0	4.13±0.21^{b}	90	0.86±0.02	180	1.5±0.09^{a}	30
Trp	1±0.00	0	2.22±0.09^{a}	120	0.94±0.03	240	1.75±0.05^{b}	60	0.51±0.03	120	1.02±0.02^{c}	15	0.8±0.04	120	1±0.00^{c}	0
Tyr	1±0.00	0	1.87±0.09^{a}	120	1±0.00	0	2.36±0.15^{b}	60	0.54±0.02	180	1.04±0.04^{c}	15	0.71±0.10	120	1±0.00^{c}	0
Val	1±0.00	0	1.66±0.09^{a}	120	1±0.00	0	2.01±0.07^{b}	90	1±0.00	0	2.59±0.08^{c}	90	0.78±0.02	120	1.04±0.07^{d}	30
Average min		**0**		**60**		**0**		**60**		**240**		**90**		**120**		**30**

Table 41: Prediabetic group: Minimum and maximum fold changes and times of amino acids after intake of different test meals

	WPI				CP				GMP				MD19			
	Min	time	Max	time	Min	time	Max	time	Min	time	Max	time	Min	time	Max	time
Aad	1.00±0.00	0	7.43±0.83[a]	180	0.96±0.04	15	3.78±0.69[b]	120	0.93±0.05	15	3.38±0.36[b]	180	0.69±0.07	120	1.00±0.00[b]	0
Abu	1.00±0.00	0	1.25±0.03[ac]	60	1.00±0.00	0	1.21±0.03[ac]	90	1.00±0.00	0	2.11±0.16[b]	240	0.75±0.01	180	1.00±0.00[c]	0
Ala	1.00±0.00	0	1.36±0.04[a]	60	1.00±0.00	0	1.47±0.04[a]	90	1.00±0.00	0	1.89±0.12[b]	90	0.98±0.02	15	1.04±0.04[c]	90
Arg	0.95±0.04	240	1.53±0.09[a]	45	1.00±0.00	0	1.60±0.06[a]	60	0.85±0.02	240	1.17±0.02[b]	60	0.76±0.02	180	1.00±0.00[b]	0
Asn	1.00±0.00	240	1.67±0.08[a]	60	0.99±0.05	240	1.77±0.07[a]	60	1.00±0.00	0	2.48±0.16[b]	60	0.79±0.02	120	1.00±0.00[c]	15
Asp	0.83±0.13	15	3.51±0.93[a]	60	1.00±0.00	0	2.68±0.39[a]	60	1.00±0.00	0	3.74±0.38[a]	120	0.86±0.04	45	1.29±0.31[b]	240
bAib	0.77±0.06	240	1.00±0.00[a]	0	0.82±0.05	180	1.00±0.00[a]	0	0.85±0.09	180	1.03±0.05[a]	15	0.79±0.06	180	1.09±0.06[a]	15
Cit	0.96±0.04	15	1.51±0.07[a]	240	0.97±0.04	15	1.41±0.06[a]	180	0.97±0.04	30	1.20±0.06[b]	120	0.62±0.03	90	1.00±0.00[b]	0
Cys	1.00±0.00	0	1.35±0.06[a]	120	0.87±0.03	240	1.05±0.05[b]	45	0.97±0.04	240	1.07±0.03[b]	45	0.93±0.03	240	1.02±0.03[b]	15
EtN	0.93±0.02	30	1.06±0.04[a]	60	0.93±0.04	15	1.05±0.05[a]	120	0.90±0.04	45	1.00±0.00[a]	0	0.94±0.04	60	1.05±0.04[a]	240
Gln	0.96±0.03	240	1.08±0.02[a]	60	0.95±0.03	240	1.13±0.02[a]	60	1.00±0.00	15	1.12±0.02[a]	60	0.92±0.02	120	1.00±0.00[b]	0
Glu	1.00±0.00	0	1.76±0.15[a]	120	0.91±0.12	240	1.63±0.14[a]	60	1.00±0.00	0	2.06±0.17[a]	120	0.71±0.08	240	1.00±0.00[b]	15
Gly	0.80±0.03	240	1.05±0.03[ab]	45	0.82±0.02	240	1.11±0.02[a]	45	0.91±0.03	240	1.11±0.03[a]	45	0.91±0.02	90	1.00±0.00[b]	0
His	0.93±0.03	240	1.18±0.04[a]	60	0.93±0.05	240	1.32±0.03[b]	60	0.90±0.02	180	1.08±0.05[ac]	30	0.89±0.01	120	1.01±0.02[c]	15
Hyp	0.80±0.02	240	1.18±0.04[a]	45	0.79±0.03	240	1.16±0.03[a]	45	0.89±0.03	240	1.46±0.14[b]	60	0.78±0.02	180	1.00±0.00[c]	0
Ile	1.00±0.00	0	3.01±0.17[a]	45	1.00±0.00	0	2.80±0.14[a]	60	1.00±0.00	0	6.23±0.62[b]	180	0.56±0.02	180	1.00±0.00[c]	0
Leu	1.00±0.00	0	3.37±0.17[a]	120	1.00±0.00	0	2.40±0.14[b]	60	0.77±0.02	240	1.42±0.05[c]	45	0.62±0.03	180	1.00±0.00[c]	15
Lys	1.00±0.00	0	2.28±0.14[a]	60	1.00±0.00	0	1.85±0.09[b]	60	1.00±0.00	0	1.76±0.11[bc]	60	0.88±0.01	120	1.01±0.02[d]	15
Met	1.00±0.00	0	2.45±0.27[a]	60	1.00±0.00	0	2.77±0.14[a]	90	1.00±0.00	0	2.30±0.19[a]	45	0.68±0.06	180	1.02±0.04[b]	15
Orn	1.00±0.00	0	1.37±0.08[ab]	60	1.00±0.00	0	1.56±0.07[a]	60	1.00±0.00	0	1.33±0.07[b]	60	0.76±0.02	120	1.01±0.03[c]	15
PEtN	0.81±0.10	180	1.14±0.11[ab]	30	0.94±0.08	15	1.31±0.09[ab]	45	1.00±0.00	0	1.52±0.11[a]	60	0.91±0.04	60	1.05±0.05[b]	15
Phe	1.00±0.00	240	1.56±0.07[a]	45	1.00±0.00	0	1.76±0.06[b]	90	0.50±0.02	180	1.06±0.02[c]	30	0.76±0.01	120	1.00±0.00[c]	0
Pro	1.00±0.00	0	1.46±0.05[a]	45	1.00±0.00	0	2.53±0.13[b]	90	1.00±0.00	0	3.04±0.27[bc]	120	0.81±0.02	240	1.00±0.00[ad]	0
Ser	0.91±0.11	240	1.45±0.13[a]	60	0.92±0.04	240	1.60±0.07[a]	60	1.00±0.00	0	2.09±0.14[b]	60	0.82±0.02	120	1.01±0.02[c]	15
Tau	0.80±0.02	180	1.01±0.03[abc]	45	0.81±0.05	180	1.03±0.09[abc]	45	0.95±0.03	240	1.12±0.03[b]	60	0.88±0.02	180	1.04±0.03[c]	15
Thr	1.00±0.00	0	1.71±0.10[a]	45	1.00±0.00	0	1.55±0.05[a]	60	1.00±0.00	0	3.77±0.25[b]	120	0.79±0.03	180	1.00±0.00[c]	0
Trp	1.00±0.00	0	2.32±0.13[a]	120	1.00±0.00	0	1.51±0.05[b]	60	0.49±0.03	180	1.00±0.00[c]	15	0.86±0.02	180	1.00±0.00[c]	15
Tyr	1.00±0.00	0	1.84±0.10[a]	120	1.00±0.00	0	2.31±0.10[b]	90	0.50±0.03	240	1.01±0.01[c]	30	0.69±0.02	180	1.00±0.00[c]	0
Val	1.00±0.00	0	1.75±0.06[a]	120	1.00±0.00	0	1.95±0.07[a]	90	1.00±0.00	0	2.81±0.17[b]	120	0.76±0.02	180	1.02±0.01[c]	15
Average min		**0**		**60**		**0**		**60**		**0**		**60**		**180**		**15**

Table 42: Overview of amino acid peaks in the healthy group and prediabetic group

Control group: BCAAs: Peaks of fold changes and times

	Val		Leu		Ile		BCAAs	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
WPI	1±0.00	1.66±0.09[a]	1±0.07	3.17±0.14[a]	0.98±0.07	2.78±0.22[a]	0.99±0.05	2.54±0.15[a]
min	0	120	0	120	0	90	0.00	120
CP	1±0.00	2.01±0.07[b]	1±0.00	2.47±0.11[b]	1±0.00	2.8±0.18[a]	1±0.00	2.43±0.12[a]
min	0	90	0	60	0	60	0	60
GMP	1±0.00	2.59±0.08[c]	0.79±0.02	1.36±0.04[c]	1±0.00	6.13±0.28[b]	1±0.00	3.36±0.13[b]
min	0	90	0	30	0	90	0	90
MD19	0.78±0.02	1.04±0.07[d]	0.61±0.08	1±0.00[d]	0.57±0.08	1.11±0.14[c]	0.65±0.06	1.05±0.07[c]
min	120	30	120	0	120	30	120	30

Control group: AAAs: Peaks of fold changes and times

	Trp		Phe		Tyr		AAAs	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
WPI	1±0.00	2.22±0.09[a]	0.84±0.03	1.55±0.06[a]	1±0.00	1.87±0.09[a]	1±0.00	1.88±0.08[a]
min	0	120	240	30	0	120	0	120
CP	0.94±0.03	2.36±0.15[b]	1±0.00	1.79±0.09[b]	1±0.00	1.75±0.05[b]	1±0.00	1.97±0.10[a]
min	240	60	0	60	0	60	0	60
GMP	0.51±0.03	1.02±0.02[c]	0.56±0.03	1.07±0.01[c]	0.54±0.02	1.04±0.04[c]	0.54±0.02	1.04±0.02[b]
min	180	15	180	15	180	15	180	15
MD19	0.8±0.04	1±0.00[c]	0.75±0.03	1±0.00[c]	0.71±0.10	1±0.00[c]	0.75±0.06	1.00[b]
min	120	0	120	0	120	0	120	0

Prediabetic group: BCAAs: Peaks of fold changes and times

	Val		Leu		Ile		BCAAs	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
WPI	1.00±0.00	1.75±0.06[a]	1.00±0.00	3.37±0.13[a]	1.00±0.00	3.01±0.17[a]	1.00±0.00	2.71±0.12[ac]
min	0	120	0	120	0	45	0	120
CP	1.00±0.00	1.95±0.07[a]	1.00±0.00	2.40±0.02[b]	1.00±0.00	2.80±0.02[a]	1.00±0.00	2.38±0.04[b]
min	0	90	0	60	0	60	0	60
GMP	1.00±0.00	2.81±0.17[b]	0.77±0.02	1.42±0.05[c]	1.00±0.00	6.23±0.62[b]	1.00±0.00	3.49±0.28[c]
min	0	120	240	45	0	180	0	120
MD19	0.76±0.02	1.02±0.01[c]	0.62±0.03	1.00±0.00[c]	0.56±0.02	1.00±0.00[c]	0.65±0.02	1.00±0.00[d]
min	180	15	180	0	180	0.00	180	0

Prediabetic group: AAAs: Peaks of fold changes and times

	Trp		Phe		Tyr		AAAs	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**
WPI	1.00±0.00	2.32±0.13[a]	1.00±0.00	1.56±0.06[a]	1.00±0.00	1.84±0.11[a]	1.00±0.00	1.90±0.1[a]
min	0	120	0	45	0	120	0	120
CP	1.00±0.00	1.51±0.05[b]	1.00±0.00	1.76±0.06[b]	1.00±0.00	2.31±0.01[b]	1.00±0.00	1.86±0.04[a]
min	0	60	0	90	0	90	0	90
GMP	0.49±0.03	1.00±0.00[c]	0.50±0.02	1.06±0.01[c]	0.50±0.03	1.01±0.01[c]	0.50±0.03	1.02±0.01[b]
min	180	15	180	30	240	30	180	30
MD19	0.86±0.02	1.00±0.00[c]	0.76±0.01	1.00±0.00[c]	0.69±0.02	1.00±0.00[c]	0.77±0.02	1.00±0.00[b]
min	180	15	120	0	180	0	180	0

Table 43: Control group: Minimum and maximum fold changes and times of plasma parameters and ΔVAS

	WPI				CP				GMP				MD19			
	Min	time	Max	time	Min	time	Max	time	Min	time	Max	time	Min	time	Max	time
Insulin	0.95±0.57[a]	240	16.84±8.84[a]	30	0.75±0.31[a]	240	15.01±11.86[a]	30	0.85±0.45[a]	240	8.40±3.80[b]	30	0.86±0.37[a]	180	11.37±8.79[ab]	30
Glucose	0.84±0.04[a]	60	1.32±0.05[a]	15	0.85±0.02[a]	60	1.28±0.03[a]	30	0.81±0.04[a]	120	1.28±0.0.04[a]	15+30	0.83±0.05[a]	120	1.58±00.07[b]	30
cont.Glucose	0.97±0.02[a]	220	1.40±0.04[a]	40	0.93±0.02[a]	140	1.34±0.03[a]	40	0.92±0.07[a]	150	1.39±0.02[a]	40	0.88±0.02[a]	150	1.61±0.05[b]	40
C-Peptide	1.00±0.00[a]	0	4.81±0.52[a]	30	0.96±0.07[a]	240	3.74±0.58[a]	30	0.76±0.05[a]	240	4.08±0.05[a]	30	0.86±0.0.06[a]	240	4.02±0.59[a]	60
GLP-1	1.00±0.00[a]	0	3.28±0.70[a]	15	1.00±0.00[a]	0	2.93±0.61[a]	15	1.00±0.00[a]	180	1.94±0.33[a]	15	1.00±0.00[a]	240	3.06±1.18[a]	15
GIP	1.00±0.00[a]	0	11.04±1.40[a]	30	1.00±0.00[a]	0	7.66±0.53[b]	30	0.78±0.10[b]	240	6.95±1.02[abc]	30	0.7±0.07[b]	240	7.06±1.07[c]	30
Glucagon	1.00±0.00[a]	0	2.07±0.17[ab]	120	1.00±0.00[a]	0	2.11±0.33[a]	60	1±0.00[a]	240	2.35±0.19[a]	90	0.67±0.08[a]	30	0.98±0.0.06[b]	180
Ghrelin	0.52±0.14[ab]	60	0.87±0.22[a]	240	0.54±0.14[a]	60	1.15±0.30[a]	240	0.41±0.11[b]	60	0.99±0.26[a]	240	0.49±0.13[ab]	60	0.97±0.25[a]	180
ΔVAS	-1.98±073[a]	60	1.97±0.42[a]	240	-1.29±0.72[a]	60	3.49±0.57[a]	240	-2.09±0.65[a]	60	2.9±0.73[a]	240	-0.4±0.50[a]	60	3.31±0.55[a]	240
Beta-HB	0.78±0.11[a]	90	1.07±0.16[a]	240	0.8±0.12[a]	30	1.26±0.29[a]	30	0.20±0.04[b]	120	1.2±0.27[a]	240	0.31±0.05[b]	90	1.47±0.35[a]	240
NEFA	0.34±0.03[a]	120	1±0.00[ab]	0	0.41±0.04[a]	90	1±0.00[a]	0	0.23±0.02[b]	120	1±0.00[a]	0	0.40±0.03[a]	90	1±0.00[a]	0

Table 44: Prediabetic group: Minimum and maximum fold changes and times of plasma parameters and Δ acetaminophen and ΔVAS

	WPI				CP				GMP				MD19			
	Min	time	Max	time	Min	time	Max	time	Min	time	Max	time	Min	time	Max	time
Insulin	1.00±0.00[a]	0	22.98±3.37[a]	60	1±0.00[a]	0	22.00±3.69[a]	90	0.85±0.12[a]	240	15.38±2.07[ab]	60	0.94±0.15[a]	240	11.48±1.67[b]	90
Glucose	0.81±0.03[a]	240	1.45±0.06[a]	45	0.74±0.03[a]	240	1.37±0.05[a]	45	0.74±0.03[a]	240	1.46±0.05[a]	30	0.72±0.02[a]	240	1.72±0.08[b]	60
cont. Glucose	0.87±0.02[a]	240	1.44±0.04[a]	40	0.80±0.02[ab]	230	1.45±0.04[a]	60	0.78±0.02[ab]	230	1.48±0.06[a]	60	0.77±0.04[b]	230	1.80±0.08[b]	70
C-Peptide	1.00±0.00[a]	0	3.65±0.27[a]	90	1±0.00[a]	0	3.64±0.24[a]	90	1±0.00[a]	0	3.50±0.31[a]	60	1±0.00[a]	0	3.42±0.25[a]	90
GLP-1 ELISA	1.00±0.00[a]	0	9.47±1.95[a]	15	1±0.00[a]	0	8.07±1.14[a]	30	1±0.00[a]	0	10.36±2.68[a]	30	0.65±0.16[b]	240	7.35±1.19[a]	45
GIP	1.00±0.00[a]	0	6.60±0.73[a]	45	1±0.00[a]	0	6.43±0.56[a]	30	1±0.00[a]	0	5.05±0.57[a]	30	1±0.00[a]	0	4.98±0.51[a]	45
Glucagon	1.00±0.00[a]	0	2.29±0.35[a]	45	1±0.00[a]	0	2.18±0.15[a]	30	1±0.00[a]	0	1.94±0.16[ab]	30	1±0.00[a]	0	1.12±0.09[b]	30
Ghrelin	0.74±0.15[a]	60	1.01±14	240	0.60±0.05[a]	90	1.04±0.08	240	0.57±0.05[a]	90	1.01±0.10	240	0.67±0.06[a]	45	1.25±0.24	240
ΔVAS	0.36±0.43[ac]	60	2.93±0.85[ab]	240	-0.99±0.6[b]	60	1.87±0.68[a]	240	0.35±0.40[abc]	60	5.49±0.65[b]	240	0.21±0.36[ac]	60	3.55±0.80[ab]	240
Beta-HB	0.69±0.08[a]	120	1±0.00[a]	0	0.62±0.08[a]	180	1.00±0.00[a]	0	0.42±0.04[b]	180	1±0.00[a]	0	0.33±0.05[b]	120	1.00±0.00[a]	0
NEFA	0.23±0.03[a]	120	1.08±0.05[a]	15	0.23±0.02[a]	120	1±0.00[a]	0	0.22±0.03[a]	120	1±0.00[a]	0	0.28±0.04[a]	120	1.02±0.04[a]	15
ΔAcetaminophen	0.00±0.00[a]	0	7.54±1.36[a]	120	0.00±0.00[a]	0	8.42±1.19[a]	120	0.00±0.00[a]	0	9.92±1.76[b]	120	0.00±0.00[a]	0	10.83±1.89[b]	90

Table 45: Control group: Correlations with insulin

Insulin	Glucose	C-Peptide	GLP-1	GIP	Ghrelin	VAS	Leu	BCAAs
WPI	0.7196	**0.9387**	0.7965	**0.828**	-0.5942	**-0.8037**	0.5758	0.5994
P value	0.0442	**0.0005**	0.018	**0.0111**	0.1204	**0.0162**	0.1353	0.1163
CP	0.6867	**0.9196**	0.86	**0.8967**	**-0.845**	**-0.8373**	0.7176	0.5768
P value	0.06	**0.0012**	0.0062	**0.0025**	**0.0083**	**0.0095**	0.0451	0.1344
GMP	0.6798	**0.9034**	0.3843	**0.7863**	-0.6595	**-0.7745**	**0.8423**	0.2215
P value	0.0636	**0.0021**	0.3472	**0.0206**	0.0752	**0.024**	**0.0087**	0.598
MD19	**0.9122**	**0.9014**	0.7837	**0.932**	-0.7679	**-0.7593**	0.2533	0.4628
P value	**0.0016**	**0.0022**	0.0214	**0.0007**	0.0261	**0.0289**	0.5449	0.2482

Table 46: Prediabetic group: Correlations with insulin

Insulin	Glucose	C-peptide	GLP-1	GIP	Glucagon	Ghrelin	BCAAs	AAAs
WPI	0.6438	**0.9673**	**0.6984**	**0.8815**	**0.7331**	**-0.9482**	**0.9179**	**0.9032**
P value	0.0613	**P<0.0001**	**0.0364**	**0.0017**	**0.0246**	**P<0.0001**	**0.0005**	**0.0008**
CP	0.7196	**0.8994**	**0.8232**	**0.8949**	**0.7775**	**-0.896**	**0.9032**	**0.9032**
P value	0.0288	**0.001**	**0.0064**	**0.0011**	**0.0136**	**0.0011**	**0.0008**	**0.0008**
GMP	0.7791	**0.9286**	**0.8915**	**0.9274**	**0.8972**	-0.7897	0.495	0.495
P value	0.0133	**0.0003**	**0.0012**	**0.0003**	**0.001**	0.0114	0.1754	0.1754
MD19	0.8572	**0.8982**	0.6447	**0.7961**	-0.1108	-0.7671	0.02075	-0.09381
P value	0.0031	**0.001**	0.0608	**0.0103**	0.7766	0.0158	0.9577	0.8103

Table 47: Control group: Correlations with C-peptide

	Glucose	Insulin	GLP-1	GIP	Ghrelin	Val	BCAAs	AAAs
WPI	0.4746	**0.9387**	0.8108	**0.9558**	-0.821	**0.7961**	**0.8268**	**0.8222**
P value	0.2347	**0.0005**	0.0146	**0.0002**	0.0125	**0.0181**	**0.0114**	**0.0122**
CP	0.3827	**0.9196**	0.7977	**0.9782**	**-0.9692**	0.6414	**0.827**	**0.884**
P value	0.3494	**0.0012**	0.0177	**P<0.0001**	**P<0.0001**	0.0865	**0.0113**	**0.0036**
GMP	0.3895	**0.9034**	0.2857	**0.9311**	**-0.8951**	0.3855	0.5224	0.4953
P value	0.3401	**0.0021**	0.4927	**0.0008**	**0.0027**	0.3457	0.1841	0.2121
MD19	0.663	**0.9014**	0.575	**0.9513**	**-0.9381**	0.3072	0.1641	0.02785
P value	0.0731	**0.0022**	0.1359	**0.0003**	**0.0006**	0.4592	0.6977	0.9478

Table 48: Prediabetic group: Correlations with C-peptide

	Glucose	Insulin	GLP-1	GIP	Glucagon	Ghrelin	BCAAs	AAAs
WPI	0.5098	**0.9673**	**0.7479**	**0.8909**	**0.833**	**-0.9277**	**0.9859**	**0.9668**
P value	0.1609	**P<0.0001**	**0.0205**	**0.0013**	**0.0053**	**0.0003**	**P<0.0001**	**P<0.0001**
CP	0.4282	**0.8994**	**0.7245**	**0.8673**	0.7779	**-0.9607**	**0.9877**	**0.9836**
P value	0.2502	**0.001**	**0.0272**	**0.0025**	0.0136	**P<0.0001**	**P<0.0001**	**P<0.0001**
GMP	0.5214	**0.9286**	**0.7391**	**0.8492**	**0.0038**	**-0.9354**	0.7435	0.2451
P value	0.15	**0.0003**	**0.0229**	**0.0038**	**0.0008**	**0.0002**	0.0217	0.5251
MD19	0.7075	**0.8982**	0.371	0.6301	-0.4976	-0.623	-0.3019	-0.4102
P value	0.033	**0.001**	0.3256	0.0689	0.1728	0.0731	0.4298	0.2728

Table 49: Control group: Correlations with glucagon

Glucagon	GIP	Ghrelin	β-HB	NEFA	BCAAs	AAAs
WPI	0.7221	**-0.9265**	-0.8272	**-0.9598**	**0.9438**	**0.9456**
P value	0.0431	**0.0009**	0.0113	**0.0002**	**0.0004**	**0.0004**
CP	0.7435	**-0.8072**	-0.7362	**-0.8616**	**0.9033**	**0.9175**
P value	0.0345	**0.0154**	0.0373	**0.006**	**0.0021**	**0.0013**
GMP	0.5312	-0.685	**-0.9179**	**-0.9236**	**0.8969**	**-0.3293**
P value	0.1755	0.0608	**0.0013**	**0.0011**	**0.0025**	**0.4257**
MD19	-0.4776	0.4972	-0.1334	-0.1228	-0.2553	-0.1233
P value	0.2313	0.21	0.7528	0.7721	0.5417	0.7712

Table 50: Prediabetic group: Correlations with glucagon

Glucagon	Glucose	Insulin	C-peptide	GLP-1	GIP	BCAAs	AAAs
WPI	0.5081	**0.7331**	**0.833**	**0.9097**	**0.8784**	**0.854**	**0.83**
P value	0.1625	**0.0246**	**0.0053**	**0.0007**	**0.0018**	**0.0034**	**0.0056**
CP	0.5329	**0.7775**	**0.7779**	**0.8792**	**0.8945**	**0.8246**	**0.857**
P value	0.1396	**0.0136**	**0.0136**	**0.0018**	**0.0011**	**0.0062**	**0.0032**
GMP	0.5542	**0.8972**	**0.9042**	**0.8765**	**0.9415**	0.6722	0.2627
P value	0.1215	**0.001**	**0.0008**	**0.0019**	**0.0002**	0.0473	0.4947
MD19	0.1951	-0.1108	-0.4976	0.4899	0.2319	**0.8681**	**0.8862**
P value	0.03806	0.01228	0.2476	0.1806	0.05379	**0.0024**	**0.0015**

Table 51: Control group: Correlations with GIP

GIP	Glucose	Insulin	C-peptide	GLP-1	Ghrelin	VAS	BCAAs	AAAs
WPI	0.3413	0.828	**0.9558**	**0.832**	**-0.8707**	-0.8249	**0.8999**	**0.9019**
P value	0.408	0.0111	**0.0002**	**0.0104**	**0.0049**	0.0117	**0.0023**	**0.0022**
CP	0.4045	**0.8967**	**0.9782**	**0.8506**	**-0.9633**	**-0.8559**	0.7938	**0.8619**
P value	0.3202	**0.0025**	**P<0.0001**	**0.0074**	**0.0001**	**0.0067**	0.0187	**0.0059**
GMP	0.4143	0.7863	**0.9311**	0.4621	-0.7899	**-0.9287**	0.5087	0.4456
P value	0.3075	0.0206	**0.0008**	0.249	0.0197	**0.0009**	0.198	0.2685
MD19	0.7599	**0.932**	**0.9513**	0.7529	**-0.8599**	**-0.8494**	0.3004	0.1765
P value	0.0287	**0.0007**	**0.0003**	0.0311	**0.0062**	**0.0076**	0.4697	0.6758

Table 52: Prediabetic group: Correlations with GIP

GIP	Glucose	Insulin	C-peptide	GLP-1	Glucagon	Ghrelin	BCAAs	AAAs
WPI	**0.7418**	**0.8815**	**0.8909**	**0.9485**	**0.8784**	**-0.8167**	**0.8371**	**0.7859**
P value	**0.0221**	**0.0017**	**0.0013**	**P<0.0001**	**0.0018**	**0.0072**	**0.0049**	**0.012**
CP	**0.7243**	**0.8949**	**0.8673**	**0.9559**	**0.8945**	**-0.8587**	**0.8682**	**0.8873**
P value	**0.0273**	**0.0011**	**0.0025**	**P<0.0001**	**0.0011**	**0.003**	**0.0024**	**0.0014**
GMP	**0.7264**	**0.9274**	**0.8492**	**0.9499**	**0.9415**	**-0.7045**	0.4506	0.5049
P value	**0.0267**	**0.0003**	**0.0038**	**P<0.0001**	**0.0002**	**0.0341**	0.2236	0.1656
MD19	**0.9462**	0.7961	0.6301	**0.9430**	0.2319	**-0.8288**	0.4435	0.3314
P value	**0.0001**	0.0103	0.0689	**P<0.0001**	0.5482	**0.0058**	0.2319	0.3836

Table 53: Control group: Correlations with GLP-1

GLP-1	Insulin	GIP
WPI	**0.7965**	0.832
P value	**0.018**	0.0104
CP	**0.86**	**0.8506**
P value	**0.0062**	**0.0074**
GMP	0.3843	0.4621
P value	0.3472	0.249
MD19	0.7837	0.7529
P value	0.0214	0.0311

Table 54: Prediabetic group: Correlations with GLP-1 (ELISA)

GLP-1	Glucose	Insulin	C-peptide	GIP	Glucagon	Ghrelin	BCAAs	AAAs
WPI	**0.6974**	**0.6984**	**0.7479**	**0.9485**	**0.9097**	-0.6176	**0.7123**	0.6618
P value	**0.0367**	**0.0364**	**0.0205**	**P<0.0001**	**0.0007**	0.0763	**0.0313**	0.0522
CP	**0.8078**	**0.8232**	**0.7245**	**0.9559**	**0.8792**	**-0.7211**	0.7453	**0.7728**
P value	**0.0084**	**0.0064**	**0.0272**	**P<0.0001**	**0.0018**	**0.0284**	0.0212	**0.0146**
GMP	**0.8083**	**0.8915**	**0.7391**	**0.9499**	**0.8765**	-0.5578	0.3293	0.5447
P value	**0.0084**	**0.0012**	**0.0229**	**P<0.0001**	**0.0019**	0.1186	0.3868	0.1294
MD19	**0.8428**	0.6447	0.371	**0.9430**	0.4899	-0.7353	0.6039	0.5084
P value	**0.0043**	0.0608	0.3256	**0.8893**	0.1806	0.024	0.085	0.1623

Table 55: Control group: Correlations with ghrelin

Ghrelin	Insulin	C-peptide	GIP	Glucagon	Beta-HB	NEFA	BCAAs	AAAs
WPI	-0.5942	**-0.821**	**-0.8707**	**-0.9265**	**0.924**	**0.8501**	**-0.9742**	**-0.975**
P value	0.1204	**0.0125**	**0.0049**	**0.0009**	**0.001**	**0.0075**	**P<0.0001**	**P<0.0001**
CP	**-0.845**	**-0.9692**	**-0.9633**	**-0.8072**	0.483	0.6249	**-0.8694**	**-0.9188**
P value	**0.0083**	**P<0.0001**	**0.0001**	**0.0154**	0.2254	0.0976	**0.005**	**0.0013**
GMP	-0.6595	**-0.8951**	-0.7899	-0.685	0.7508	0.7174	-0.7144	-0.2913
P value	0.0752	**0.0027**	0.0197	0.0608	0.0318	0.0451	0.0465	0.484
MD19	-0.7679	**-0.9381**	-0.8599	0.4972	0.7319	0.7235	0.1294	0.259
P value	0.0261	**0.0006**	0.0062	0.21	0.039	0.0425	0.7602	0.5357

Table 56: Control group: Correlations with NEFA

NEFA	Glucagon	Ghrelin	β-HB	BCAAs	AAAs
WPI	**-0.9598**	**0.8501**	**0.8071**	**-0.8346**	**-0.8376**
P value	**0.0002**	**0.0075**	**0.0155**	**0.01**	**0.0094**
CP	**-0.8616**	0.6249	**0.8651**	**-0.8929**	**-0.8482**
P value	**0.006**	0.0976	**0.0055**	**0.0028**	**0.0078**
GMP	**-0.9236**	0.7174	**0.9894**	**-0.9527**	0.4158
P value	**0.0011**	0.0451	**P<0.0001**	**0.0003**	0.3056
MD19	-0.1228	0.7235	**0.9829**	0.5768	0.6099
P value	0.7721	0.0425	**P<0.0001**	0.1344	0.1084

Table 57: Prediabetic group: Correlations with NEFA

NEFA	β-HB	Acetaminophen	BCAAs	AAAs
WPI	**0.9746**	**-0.9156**	-0.7594	**-0.8185**
P value	**P<0.0001**	**0.0005**	0.0176	**0.007**
CP	**0.9745**	**-0.942**	-0.7163	-0.7065
P value	**P<0.0001**	**0.0001**	0.03	0.0334
GMP	**0.9895**	**-0.9364**	**-0.9404**	0.4865
P value	**P<0.0001**	**0.0002**	**0.0002**	0.1841
MD19	**0.9561**	**-0.8285**	**0.8674**	**0.9119**
P value	**P<0.0001**	**0.0058**	**0.0025**	**0.0006**

Table 58: Control group: Correlations with β-HB

β -HB	Glucagon	Ghrelin	NEFA	BCAAs	AAAs
WPI	**-0.8272**	**0.924**	**0.8071**	**-0.8363**	**-0.8458**
P value	**0.0113**	**0.001**	**0.0155**	**0.0097**	**0.0081**
CP	**-0.7362**	0.483	**0.8651**	**-0.8363**	**-0.8458**
P value	**0.0373**	0.2254	**0.0055**	**0.0097**	**0.0081**
GMP	**-0.9179**	0.7508	**0.9894**	**-0.9325**	0.3317
P value	**0.0013**	0.0318	**P<0.0001**	**0.0007**	0.4222
MD19	-0.1334	0.7319	**0.9829**	0.4828	0.5266
P value	0.7528	0.039	**P<0.0001**	0.2256	0.18

Table 59: Prediabetic group: Correlations with β-HB

β -HB	NEFA	Acetaminophen	BCAAs	AAAs
WPI	**0.9746**	**-0.9668**	**-0.8547**	**-0.8985**
P value	**P<0.0001**	**P<0.0001**	**0.0033**	**0.001**
CP	**0.9745**	**-0.8881**	-0.5649	-0.5543
P value	**P<0.0001**	**0.0014**	0.113	0.1214
GMP	**0.9895**	**-0.9217**	**-0.9132**	0.5391
P value	**P<0.0001**	**0.0004**	**0.0006**	0.1342
MD19	**0.9561**	**-0.8914**	0.7329	**0.8068**
P value	**P<0.0001**	**0.0012**	0.0247	**0.0086**

Table 60: Prediabetic group: Correlations with insulin and amino acids

Insulin	WPI	P value	CP	P value	GMP	P value
Abu	**0.9534**	**P<0.0001**	**0.9765**	**P<0.0001**	-0.1571	0.6865
Ala	**0.9448**	**0.0001**	**0.87**	**0.0023**	0.6242	0.0724
Arg	**0.9582**	**P<0.0001**	**0.9695**	**P<0.0001**	**0.9319**	**0.0003**
Asn	0.9735	**P<0.0001**	**0.9807**	**P<0.0001**	**0.9017**	**0.0009**
Asp	**0.8208**	**0.0067**	**0.9005**	**0.0009**	0.5838	0.0988
Cys	**0.8995**	**0.001**	0.6516	0.0573	0.8664	0.0025
Gln	**0.9317**	**0.0003**	**0.9257**	**0.0003**	**0.8517**	**0.0036**
Glu	**0.9053**	**0.0008**	**0.9105**	**0.0006**	0.6706	0.048
Gly	0.4027	0.2826	0.6512	0.0575	**0.9224**	**0.0004**
His	**0.9402**	**0.0002**	**0.9429**	**0.0001**	0.7013	0.0353
Ile	**0.9665**	**P<0.0001**	**0.9622**	**P<0.0001**	0.4686	0.2033
Leu	**0.8923**	**0.0012**	**0.9614**	**P<0.0001**	0.9402	0.0002
Lys	**0.9513**	**P<0.0001**	**0.9796**	**P<0.0001**	**0.874**	**0.0021**
Met	**0.9687**	**P<0.0001**	**0.9295**	**0.0003**	**0.896**	**0.0011**
Orn	**0.887**	**0.0014**	**0.8871**	**0.0014**	**0.9348**	**0.0002**
Phe	**0.9631**	**P<0.0001**	**0.9533**	**P<0.0001**	0.5222	0.1492
Pro	**0.9578**	**P<0.0001**	0.7429	0.0218	0.523	0.1485
Ser	0.863	**0.0027**	**0.9728**	**P<0.0001**	**0.8869**	**0.0014**
Thr	**0.9612**	**P<0.0001**	**0.9749**	**P<0.0001**	0.4511	0.2229
Trp	0.7192	0.029	0.9703	P<0.0001	0.4438	0.2314
Tyr	**0.8259**	**0.0061**	**0.7886**	**0.0115**	0.4994	0.171
Val	**0.9015**	**0.0009**	0.7908	0.0112	0.3759	0.3187

Table 61: Control group: Correlations with C-peptide and amino acids

C-peptide	WPI	P value	CP	P value	GMP	P value
Abu	**0.8427**	**0.0086**	**0.935**	**0.0007**	0.1915	0.6496
Ala	0.6154	0.1044	0.7473	0.0331	0.6036	0.1131
Arg	**0.9216**	**0.0011**	**0.9809**	**P<0.0001**	**0.93**	**0.0008**
Asn	**0.8973**	**0.0025**	**0.9303**	**0.0008**	**0.8523**	**0.0072**
Asp	0.7777	0.0231	0.8222	0.0122	0.691	0.0577
Cys	0.7681	0.026	0.8794	0.004	**0.8774**	**0.0042**
Gln	**0.8845**	**0.0035**	**0.726**	**0.0414**	**0.7939**	**0.0187**
Glu	0.8053	0.0159	**0.934**	**0.0007**	0.7874	0.0203
Gly	**0.8024**	**0.0166**	**0.9375**	**0.0006**	**0.9025**	**0.0022**
His	**0.9034**	**0.0021**	**0.9186**	**0.0013**	0.7731	0.0245
Ile	**0.8835**	**0.0036**	**0.9108**	**0.0017**	0.474	0.2353
Leu	**0.8067**	**0.0155**	**0.9229**	**0.0011**	0.922	0.0011
Lys	0.8187	0.013	**0.9426**	**0.0005**	0.834	0.0101
Met	**0.9374**	**0.0006**	**0.9542**	**0.0002**	**0.9228**	**0.0011**
Orn	**0.8793**	**0.004**	**0.7558**	**0.0301**	**0.9641**	**0.0001**
Phe	**0.93**	**0.0008**	**0.9514**	**0.0003**	0.5093	0.1973
Pro	**0.8515**	**0.0073**	0.6981	0.0542	0.6137	0.1056
Ser	**0.9417**	**0.0005**	**0.9639**	**0.0001**	**0.8723**	**0.0047**
Tau	0.3551	0.3881	0.7384	0.0364	0.7732	0.0244
Thr	0.8272	0.0113	0.8516	0.0073	0.5262	0.1804
Trp	0.6508	0.0805	**0.9624**	**0.0001**	0.4135	0.3085
Tyr	0.8103	0.0147	0.7672	0.0263	0.5346	0.1722
Val	0.7961	0.0181	0.6414	0.0865	0.3855	0.3457

Table 62: Prediabetic group: Correlations with C-peptide and amino acids

C-peptide	WPI	P value	CP	P value	GMP	P value
Abu	**0.9596**	**P<0.0001**	**0.9168**	**0.0005**	0.08845	0.821
Ala	**0.9585**	**P<0.0001**	**0.9805**	**P<0.0001**	**0.8418**	**0.0044**
Arg	**0.9165**	**0.0005**	**0.897**	**0.001**	**0.851**	**0.0036**
Asn	**0.914**	**0.0006**	**0.8623**	**0.0028**	**0.9877**	**P<0.0001**
Asp	**0.8464**	**0.004**	**0.8494**	**0.0038**	**0.8235**	**0.0064**
Cys	**0.9589**	**P<0.0001**	**0.4257**	**0.2532**	**0.9163**	**0.0005**
Gln	**0.8631**	**0.0027**	**0.806**	**0.0087**	**0.968**	**P<0.0001**
Glu	**0.962**	**P<0.0001**	**0.9339**	**0.0002**	**0.8853**	**0.0015**
Gly	0.2042	0.5981	0.3461	0.3616	0.8314	0.0055
Ile	**0.9973**	**P<0.0001**	**0.979**	**P<0.0001**	0.708	0.0328
Leu	**0.9735**	**P<0.0001**	**0.9703**	**P<0.0001**	**0.8121**	**0.0078**
Lys	**0.9955**	**P<0.0001**	**0.9512**	**P<0.0001**	**0.979**	**P<0.0001**
Met	**0.9764**	**P<0.0001**	**0.9694**	**P<0.0001**	**0.9771**	**P<0.0001**
Orn	**0.945**	**0.0001**	**0.9667**	**P<0.0001**	**0.8619**	**0.0028**
Phe	**0.9402**	**0.0002**	**0.964**	**P<0.0001**	0.2638	0.4927
Pro	**0.9979**	**P<0.0001**	**0.9253**	**0.0003**	**0.7562**	**0.0184**
Ser	0.7425	0.0219	**0.8376**	**0.0048**	**0.9842**	**P<0.0001**
Thr	**0.9941**	**P<0.0001**	**0.966**	**P<0.0001**	0.6935	0.0383
Trp	**0.8511**	**0.0036**	**0.9607**	**P<0.0001**	0.1923	0.6201
Tyr	**0.9313**	**0.0003**	**0.9419**	**0.0001**	0.2626	0.4949
Val	**0.9768**	**P<0.0001**	**0.9507**	**P<0.0001**	0.6227	0.0733

Table 63: Control group: Correlations with glucagon

Glucagon	WPI	P value	CP	P value	GMP	P value
Ala	**0.9719**	**P<0.0001**	**0.8392**	**0.0092**	**0.902**	**0.0022**
Arg	0.7985	0.0175	**0.8424**	**0.0087**	0.5322	0.1745
Asn	**0.8624**	**0.0059**	**0.835**	**0.0099**	**0.789**	**0.0199**
Asp	**0.9104**	**0.0017**	**0.7278**	**0.0407**	**0.8704**	**0.0049**
Cys	**0.9565**	**0.0002**	0.5887	0.1247	0.2692	0.5192
Glu	**0.9003**	**0.0023**	0.8284	0.0111	**0.8386**	**0.0093**
Hyp	**0.8763**	**0.0043**	0.7411	0.0354	0.7207	0.0437
Ile	**0.891**	**0.003**	**0.8932**	**0.0028**	**0.905**	**0.002**
Leu	**0.9501**	**0.0003**	**0.8977**	**0.0025**	0.2788	0.5037
Lys	**0.9435**	**0.0004**	**0.8532**	**0.0071**	**0.8354**	**0.0098**
Orn	**0.8749**	**0.0044**	**0.8905**	**0.003**	0.6037	0.113
Phe	0.7203	0.0439	**0.8833**	**0.0036**	-0.3289	0.4263
Pro	**0.9273**	**0.0009**	**0.8604**	**0.0061**	**0.9231**	**0.0011**
Thr	**0.9396**	**0.0005**	**0.8596**	**0.0062**	**0.9094**	**0.0017**
Trp	**0.9815**	**P<0.0001**	**0.8729**	**0.0047**	-0.4191	0.3013
Tyr	**0.9516**	**0.0003**	**0.9028**	**0.0021**	-0.2632	0.5288
Val	**0.9563**	**0.0002**	**0.851**	**0.0074**	**0.8637**	**0.0057**

Table 64: Prediabetic group: Correlations with glucagon and amino acids

Glucagon	WPI	P value	CP	P value	GMP	P value	MD19	P value
Aad	0.2442	0.5266	0.2842	0.4587	-0.002522	0.9949	**0.9619**	**P<0.0001**
Abu	**0.8288**	**0.0058**	**0.7327**	**0.0247**	0.05315	0.892	**0.8051**	**0.0088**
Arg	0.7699	0.0152	**0.8248**	**0.0062**	0.796	0.0103	**0.8678**	**0.0024**
Asn	0.7277	0.0263	0.7399	0.0226	**0.8803**	**0.0017**	**0.9005**	**0.0009**
Cit	0.2299	0.5518	0.3075	0.4209	-0.1129	0.7723	**0.8197**	**0.0068**
Gln	0.5365	0.1365	0.5611	0.116	0.7887	0.0115	**0.8119**	**0.0079**
Glu	**0.8205**	**0.0067**	0.6423	0.0621	0.7606	0.0173	0.3994	0.2869
His	0.562	0.1152	0.6416	0.0625	0.4329	0.2445	**0.8691**	**0.0023**
Hyp	0.4085	0.275	0.4188	0.2619	**0.8527**	**0.0035**	**0.8105**	**0.0081**
Ile	**0.8585**	**0.003**	**0.8238**	**0.0063**	0.6287	0.0697	**0.8568**	**0.0032**
Leu	**0.8507**	**0.0036**	**0.8541**	**0.0034**	0.7872	0.0118	**0.8836**	**0.0016**
Met	**0.8246**	**0.0062**	**0.8318**	**0.0054**	**0.9584**	**P<0.0001**	**0.827**	**0.006**
Orn	**0.8458**	**0.0041**	**0.824**	**0.0063**	**0.8449**	**0.0041**	**0.8805**	**0.0017**
Phe	**0.7955**	**0.0104**	**0.8666**	**0.0025**	0.2891	0.4505	**0.913**	**0.0006**
Pro	**0.8532**	**0.0034**	0.7224	0.0279	0.6641	0.0511	0.7936	0.0107
Ser	0.5668	0.1115	0.7163	0.03	**0.8934**	**0.0012**	**0.8942**	**0.0011**
Tau	-0.1007	0.7966	0.005353	0.9891	**0.8263**	**0.006**	0.6395	0.0637
Thr	0.8388	0.0047	0.7723	0.0147	0.5956	0.0906	**0.8942**	**0.0011**
Trp	0.7252	0.027	**0.8453**	**0.0041**	0.2041	0.5983	**0.8605**	**0.0029**
Tyr	**0.8162**	**0.0073**	**0.7996**	**0.0097**	0.2751	0.4737	**0.8563**	**0.0032**
Val	**0.8375**	**0.0048**	0.7528	0.0192	0.562	0.1153	**0.8593**	**0.003**

Table 65: Control group: Correlations with GIP and amino acids

GIP	WPI	P value	CP	P value	GMP	P value
Abu	**0.8667**	**0.0053**	**0.8955**	**0.0026**	0.1668	0.6929
Arg	**0.9685**	**P<0.0001**	**0.9568**	**0.0002**	**0.8814**	**0.0038**
Asn	**0.95**	**0.0003**	**0.8815**	**0.0038**	0.7846	0.0211
Gln	**0.9098**	**0.0017**	0.634	0.0914	0.6882	0.0592
Glu	**0.8602**	**0.0061**	**0.9008**	**0.0023**	0.7439	0.0343
Gly	0.7677	0.0261	**0.8916**	**0.0029**	**0.8467**	**0.008**
His	**0.9258**	**0.001**	**0.842**	**0.0087**	0.6731	0.0673
Ile	**0.942**	**0.0005**	**0.8725**	**0.0047**	0.4647	0.246
Leu	**0.8846**	**0.0035**	**0.9007**	**0.0023**	**0.8601**	**0.0061**
Lys	**0.8975**	**0.0025**	**0.8977**	**0.0025**	0.7932	0.0188
Met	**0.9602**	**0.0002**	**0.9214**	**0.0011**	**0.9012**	**0.0022**
Orn	**0.9375**	**0.0006**	0.7234	**0.0425**	**0.8659**	0.0054
Phe	**0.9717**	**P<0.0001**	**0.9365**	**0.0006**	0.4583	0.2534
Pro	**0.9108**	**0.0017**	0.6612	0.0742	0.5921	0.122
Ser	**0.9689**	**P<0.0001**	**0.9208**	**0.0012**	0.8029	0.0164
Thr	**0.8941**	**0.0027**	0.7975	0.0177	0.508	0.1987
Trp	**0.7472**	0.0331	**0.9497**	**0.0003**	0.3601	0.381
Tyr	**0.8905**	**0.003**	0.7389	0.0362	0.4893	0.2184
Val	**0.8716**	**0.0048**	0.604	0.1128	0.3807	0.3521

Table 66: Prediabetic group: Correlations with GIP and amino acids

GIP	WPI	P value	CP	P value	GMP	P value
Abu	**0.8813**	**0.0017**	**0.9142**	**0.0006**	-0.2166	0.5756
Arg	**0.9365**	**0.0002**	**0.9525**	**P<0.0001**	**0.8877**	**0.0014**
Asn	**0.9077**	**0.0007**	**0.9037**	**0.0008**	0.7915	0.011
Gly	0.4369	0.2397	0.5916	0.0934	**0.8515**	**0.0036**
His	**0.8093**	**0.0082**	**0.8155**	**0.0074**	0.6226	0.0733
Hyp	0.7288	0.0259	0.6999	0.0358	**0.8758**	**0.002**
Ile	**0.9078**	**0.0007**	**0.9179**	**0.0005**	**0.3993**	0.287
Leu	**0.8095**	**0.0082**	**0.9281**	**0.0003**	**0.8877**	**0.0014**
Lys	**0.8607**	**0.0029**	**0.9198**	**0.0004**	**0.7982**	**0.0099**
Met	**0.9271**	**0.0003**	**0.9024**	**0.0009**	**0.8692**	**0.0023**
Orn	0.7749	0.0142	**0.8397**	**0.0046**	**0.817**	**0.0072**
Phe	**0.9483**	**P<0.0001**	**0.9592**	**P<0.0001**	0.5295	0.1426
Pro	**0.8905**	**0.0013**	0.6903	0.0395	0.4487	0.2257
Ser	**0.8113**	**0.0079**	**0.8923**	**0.0012**	0.7907	0.0112
Tau	0.2275	0.5561	0.3158	0.4078	**0.8642**	**0.0027**
Thr	**0.8646**	**0.0026**	**0.8861**	**0.0015**	0.3663	0.3323
Trp	0.592	0.0931	**0.9476**	**0.0001**	0.4529	0.2209
Val	0.807	0.0086	0.7375	0.0233	0.3174	0.4053

Table 67: Control group: Correlations with ghrelin and amino acids

Ghrelin	WPI	P value	CP	P value	GMP	P value
Abu	-0.7512	0.0317	**-0.9151**	**0.0014**	-0.4406	0.2745
Ala	**-0.9176**	**0.0013**	-0.7781	0.023	**-0.848**	**0.0078**
Arg	**-0.8961**	**0.0026**	**-0.9778**	**P<0.0001**	**-0.9234**	**0.0011**
Asn	**-0.9405**	**0.0005**	**-0.9289**	**0.0008**	**-0.9722**	**P<0.0001**
Asp	**-0.909**	**0.0018**	**-0.8545**	**0.0069**	**-0.9094**	**0.0017**
Cys	**-0.9844**	**P<0.0001**	-0.8027	0.0165	**-0.8429**	**0.0086**
Glu	**-0.9231**	**0.0011**	**-0.9487**	**0.0003**	**-0.9574**	**0.0002**
Gly	-0.4767	0.2324	-0.874	0.0045	**-0.9451**	**0.0004**
His	**-0.8609**	**0.006**	**-0.9059**	**0.0019**	-0.6039	0.1129
Ile	**-0.9552**	**0.0002**	**-0.9279**	**0.0009**	-0.6844	0.0611
Leu	**-0.9749**	**P<0.0001**	**-0.948**	**0.0003**	-0.8241	0.0119
Lys	**-0.9683**	**P<0.0001**	**-0.9439**	**0.0004**	**-0.9599**	**0.0002**
Met	**-0.9278**	**0.0009**	**-0.961**	**0.0001**	**-0.9615**	**0.0001**
Orn	**-0.9669**	**P<0.0001**	-0.8209	0.0125	**-0.9716**	**P<0.0001**
Phe	**-0.841**	**0.0089**	**-0.9637**	**0.0001**	-0.2972	0.4747
Pro	**-0.9762**	**P<0.0001**	-0.7525	0.0312	-0.8181	0.0131
Ser	**-0.8335**	**0.0102**	**-0.9496**	**0.0003**	**-0.9776**	**P<0.0001**
Thr	**-0.9726**	**P<0.0001**	**-0.8633**	**0.0057**	-0.7417	0.0352
Trp	**-0.9489**	**0.0003**	**-0.9712**	**P<0.0001**	-0.1994	0.6359
Tyr	**-0.9729**	**P<0.0001**	-0.8228	0.0121	-0.347	0.3997
Val	**-0.9679**	**P<0.0001**	-0.7097	0.0486	-0.5898	0.1238

Table 68: Prediabetic group: Correlations with ghrelin and amino acids

Ghrelin	WPI	P value	CP	P value	GMP	P value
Abu	-0.03829	0.9221	**-0.9397**	**0.0002**	-0.03829	0.9221
Ala	**-0.8078**	**0.0084**	**-0.934**	**0.0002**	**-0.8078**	**0.0084**
Arg	**-0.8058**	**0.0087**	**-0.9002**	**0.0009**	**-0.8058**	**0.0087**
Asn	**-0.9117**	**0.0006**	**-0.8853**	**0.0015**	**-0.9117**	**0.0006**
Asp	**-0.8487**	**0.0038**	**-0.8364**	**0.0049**	**-0.8487**	**0.0038**
Cys	**-0.9278**	**0.0003**	**-0.6262**	**0.0712**	**-0.9278**	**0.0003**
Gln	**-0.9497**	**P<0.0001**	**-0.8932**	**0.0012**	**-0.9497**	**P<0.0001**
Glu	**-0.9023**	**0.0009**	**-0.9234**	**0.0004**	**-0.9023**	**0.0009**
His	-0.2936	0.4432	**-0.8053**	**0.0088**	-0.2936	0.4432
Hyp	**-0.8462**	**0.004**	**-0.68**	**0.0439**	**-0.8462**	**0.004**
Ile	-0.6582	0.0539	**-0.9463**	**0.0001**	-0.6582	0.0539
Leu	-0.7175	0.0295	**-0.924**	**0.0004**	-0.7175	0.0295
Lys	**-0.9013**	**0.0009**	**-0.922**	**0.0004**	**-0.9013**	**0.0009**
Met	**-0.8924**	**0.0012**	**-0.9191**	**0.0005**	**-0.8924**	**0.0012**
Orn	-0.6546	0.0557	**-0.8748**	**0.002**	-0.6546	0.0557
Phe	-0.2253	0.5599	**-0.9352**	**0.0002**	-0.2253	0.5599
Pro	-0.7131	0.031	**-0.7995**	**0.0097**	-0.7131	0.031
Ser	**-0.9025**	**0.0009**	**-0.873**	**0.0021**	**-0.9025**	**0.0009**
Thr	-0.6414	0.0626	**-0.9507**	**P<0.0001**	-0.6414	0.0626
Trp	-0.1831	0.6373	**-0.9408**	**0.0002**	-0.1831	0.6373
Tyr	-0.2608	0.498	**-0.8199**	**0.0068**	-0.2608	0.498
Val	-0.5647	0.1132	**-0.8378**	**0.0048**	-0.5647	0.1132

Table 69: Control group: Correlations with NEFA and amino acids

NEFA	WPI	P value	CP	P value	GMP	P value
Aad	**-0.8046**	**0.016**	**-0.8421**	**0.0087**	**-0.8574**	**0.0065**
Ala	**-0.9393**	**0.0005**	**-0.9082**	**0.0018**	**-0.9519**	**0.0003**
Asp	-0.8054	0.0158	-0.7751	0.0238	**-0.8699**	**0.005**
Cys	**-0.8767**	**0.0043**	-0.5092	0.1975	-0.2705	0.517
Glu	-0.7709	0.0251	-0.6531	0.0791	-0.8416	0.0088
Ile	-0.7456	0.0337	-0.8291	0.0109	**-0.9625**	**0.0001**
Leu	**-0.858**	**0.0064**	-0.8028	0.0164	-0.2274	0.5881
Lys	**-0.8371**	**0.0095**	-0.7595	0.0288	-0.8491	0.0076
Orn	-0.7391	0.0362	**-0.9315**	**0.0008**	-0.6606	0.0746
Pro	**-0.8117**	**0.0144**	**-0.935**	**0.0007**	**-0.9601**	**0.0002**
Thr	-0.8325	**0.0103**	**-0.8737**	**0.0046**	**-0.9522**	**0.0003**
Trp	**-0.9558**	**0.0002**	-0.7162	0.0457	0.5057	0.2011
Tyr	**-0.8497**	**0.0076**	**-0.9001**	**0.0023**	0.3453	0.4022
Val	**-0.8509**	**0.0074**	**-0.9432**	**0.0004**	**-0.9313**	**0.0008**

Table 70: Prediabetic group: Correlations with NEFAs and amino acids

NEFA	WPI	P value	CP	P value	GMP	P value	MD19	P value
Aad	**-0.8811**	**0.0017**	**-0.9688**	**P<0.0001**	-0.7451	0.0212	**0.9065**	**0.0008**
Abu	-0.5409	0.1326	-0.3971	0.2899	-0.6733	0.0468	**0.8023**	**0.0093**
Ala	**-0.723**	**0.0277**	**-0.759**	**0.0177**	**-0.9331**	**0.0002**	-0.6556	0.0552
Arg	-0.3191	0.4027	-0.3546	0.3491	-0.2479	0.5201	**0.8986**	**0.001**
Asn	-0.3265	0.3912	-0.2959	0.4395	-0.7242	0.0274	**0.9352**	**0.0002**
Asp	**-0.7708**	**0.015**	-0.4911	0.1794	**-0.9097**	**0.0007**	0.1568	0.687
Cit	**-0.8642**	**0.0027**	**-0.8815**	**0.0017**	-0.5699	0.1091	**0.9683**	**P<0.0001**
Gln	-0.3611	0.3397	-0.2233	0.5635	-0.7245	0.0273	**0.8824**	**0.0016**
Glu	-0.7791	0.0133	-0.6142	0.0785	**-0.8961**	**0.0011**	0.596	0.0903
Gly	0.5578	0.1186	0.3878	0.3023	-0.202	0.6021	**0.8123**	**0.0078**
His	-0.2596	0.5	-0.1356	0.7279	0.3586	0.3432	**0.9074**	**0.0007**
Ile	-0.625	0.0719	-0.5868	0.0967	**-0.9542**	**P<0.0001**	**0.8706**	**0.0023**
Leu	**-0.7955**	**0.0104**	-0.5805	0.1013	-0.138	0.7233	**0.8836**	**0.0016**
Lys	-0.7027	0.0348	-0.5152	0.1558	-0.7882	0.0116	**0.9839**	**P<0.0001**
Met	-0.5166	0.1545	-0.6047	0.0845	-0.7197	0.0288	**0.9396**	**0.0002**
Orn	-0.7567	0.0183	-0.7365	0.0236	-0.4268	0.252	**0.8819**	**0.0017**
Phe	-0.3719	0.3244	-0.5341	0.1386	0.4844	0.1863	**0.9528**	**P<0.0001**
Pro	-0.6592	0.0534	**-0.9017**	**0.0009**	**-0.9619**	**P<0.0001**	**0.8262**	**0.0061**
Ser	-0.04662	0.9052	-0.2533	0.5107	-0.7443	0.0214	**0.9699**	**P<0.0001**
Thr	-0.6821	0.043	-0.5625	0.1149	**-0.9475**	**0.0001**	**0.9012**	**0.0009**
Trp	**-0.9481**	**P<0.0001**	-0.5162	0.1548	0.527	0.1449	**0.8409**	**0.0045**
Tyr	**-0.8721**	**0.0022**	**-0.8436**	**0.0043**	0.4539	0.2197	**0.8883**	**0.0014**
Val	**-0.7941**	**0.0106**	**-0.8686**	**0.0024**	**-0.9305**	**0.0003**	**0.8497**	**0.0037**

Table 71: Control group: Correlations with β-HB and amino acids

β -HB	WPI	P value	CP	P value	GMP	P value	MD19	P value
PEtN	-0.421	0.299	-0.421	0.299	-0.7892	0.0199	**0.89**	**0.0031**
Asn	-0.7655	0.0268	-0.7655	0.0268	**-0.8355**	**0.0098**	-0.1527	0.7182
Asp	-0.7249	0.0419	-0.7249	0.0419	**-0.8899**	**0.0031**	0.7351	0.0377
EtN	-0.03971	0.9256	-0.03971	0.9256	0.07222	0.8651	**-0.8851**	**0.0035**
Ala	-0.769	0.0257	-0.769	0.0257	**-0.9395**	**0.0005**	**-0.8911**	**0.003**
Thr	-0.8175	0.0132	-0.8175	0.0132	**-0.9303**	**0.0008**	-0.07781	0.8547
Glu	**-0.7827**	**0.0217**	**-0.7827**	**0.0217**	**-0.8757**	**0.0044**	-0.0996	0.8145
His	-0.6603	0.0747	-0.6603	**0.0747**	**0.03011**	0.9436	**0.8387**	**0.0093**
Pro	**-0.8301**	**0.0108**	**-0.8301**	**0.0108**	**-0.9537**	**0.0002**	-0.1354	0.7492
Arg	-0.7352	0.0377	-0.7352	0.0377	-0.5746	0.1363	0.671	0.0685
Orn	**-0.8791**	**0.004**	**-0.8791**	**0.004**	-0.7112	0.0479	0.1475	0.7274
Cys	**-0.8732**	**0.0046**	**-0.8732**	**0.0046**	-0.3401	0.4098	-0.15	0.723
Lys	**-0.8152**	**0.0137**	**-0.8152**	**0.0137**	**-0.8766**	**0.0043**	**0.8045**	**0.016**
Val	**-0.8323**	**0.0104**	**-0.8323**	**0.0104**	**-0.8961**	**0.0026**	0.3378	0.4132
Tyr	**-0.8401**	**0.009**	**-0.8401**	**0.009**	0.2534	0.5449	0.485	0.2231
Ile	**-0.8061**	**0.0157**	**-0.8061**	**0.0157**	**-0.9398**	**0.0005**	0.4442	0.2702
Leu	**-0.843**	**0.0086**	**-0.843**	**0.0086**	-0.304	0.4642	0.6588	0.0756
Phe	-0.681	0.063	-0.681	0.063	0.3392	0.4111	0.6752	0.0662
Trp	**-0.8617**	**0.0059**	**-0.8617**	**0.0059**	0.4292	0.2886	0.3831	0.3488

Table 72: Prediabetic group: Correlations with β-HB and amino acids

β-HB	WPI	P value	CP	P value	GMP	P value	MD19	P value
Aad	**-0.8046**	**0.0089**	**-0.9888**	**P<0.0001**	-0.7873	0.0118	**0.8618**	**0.0028**
Ala	-0.786	0.012	-0.6162	0.0772	**-0.9001**	**0.0009**	-0.6935	0.0383
Asn	-0.465	0.2072	-0.1036	0.7909	-0.6694	0.0486	**0.8297**	**0.0057**
Asp	**-0.8165**	**0.0072**	**-0.3283**	**0.3884**	**-0.8476**	**0.0039**	0.3037	0.4269
Cit	-0.7937	0.0107	**-0.9453**	**0.0001**	-0.5938	0.0918	**0.9352**	**0.0002**
Cys	**-0.8527**	**0.0035**	0.3928	0.2956	-0.4602	0.2125	0.4603	0.2125
Gln	-0.4518	0.2221	-0.04	0.9186	-0.6742	0.0464	**0.9265**	**0.0003**
Glu	**-0.8512**	**0.0036**	-0.4519	0.222	**-0.8411**	**0.0045**	0.4195	0.261
Gly	0.4496	0.2247	0.5544	0.1214	-0.1249	0.7488	0.8661	0.0025
His	-0.3665	0.332	0.0441	0.9103	0.4182	0.2627	**0.9048**	**0.0008**
Ile	-0.7431	0.0218	-0.4142	0.2677	**-0.9335**	**0.0002**	0.7388	0.023
Leu	**-0.8854**	**0.0015**	-0.4089	0.2745	-0.06247	0.8732	0.7565	0.0183
Lys	**-0.8042**	**0.009**	-0.3404	0.3701	-0.7398	0.0227	**0.9138**	**0.0006**
Met	-0.6372	0.0649	-0.4371	0.2394	-0.6523	0.0569	**0.8444**	**0.0042**
Orn	**-0.8449**	**0.0041**	-0.592	0.0931	-0.3749	0.3202	0.7405	0.0225
Phe	-0.5118	0.159	-0.3534	0.3508	0.5395	0.1338	**0.8822**	**0.0016**
Pro	**-0.77**	**0.0152**	**-0.8015**	**0.0094**	**-0.9404**	**0.0002**	0.6711	0.0478
Ser	-0.1926	0.6195	-0.05991	0.8783	-0.6828	0.0427	**0.9037**	**0.0008**
Thr	-0.7908	0.0112	-0.3929	0.2956	**-0.9287**	**0.0003**	0.7936	0.0107
Trp	**-0.9836**	**P<0.0001**	-0.336	0.3767	0.5769	0.1038	0.7016	0.0352
Tyr	**-0.939**	**0.0002**	-0.7302	0.0255	0.5051	0.1655	0.7696	0.0153
Val	**-0.8772**	**0.0019**	-0.7558	0.0185	**-0.9129**	**0.0006**	0.7079	0.0329

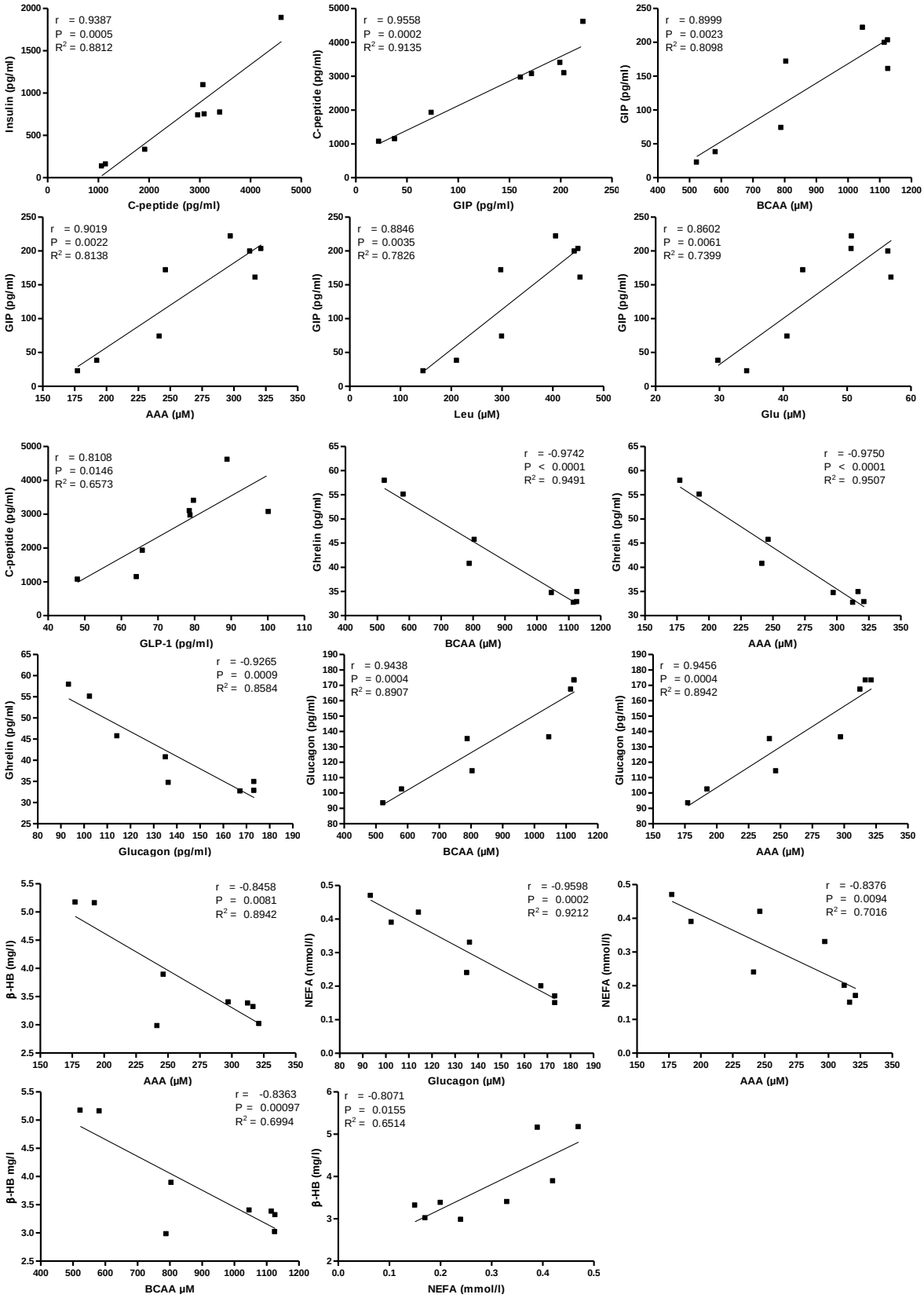


Figure 44: Correlations after intake of the WPI drink in the healthy group

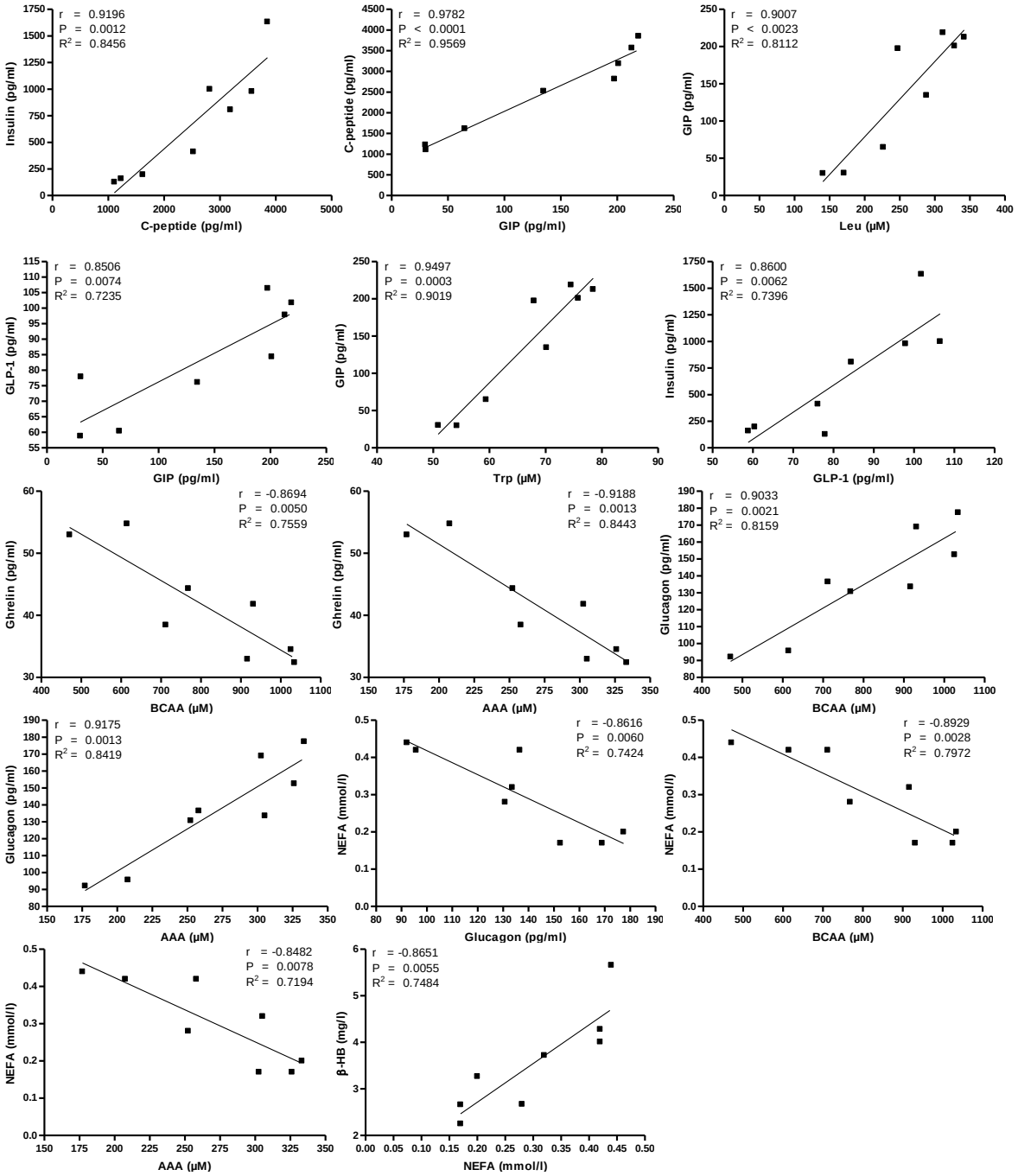


Figure 45: Correlations after intake of the CP drink in the healthy group

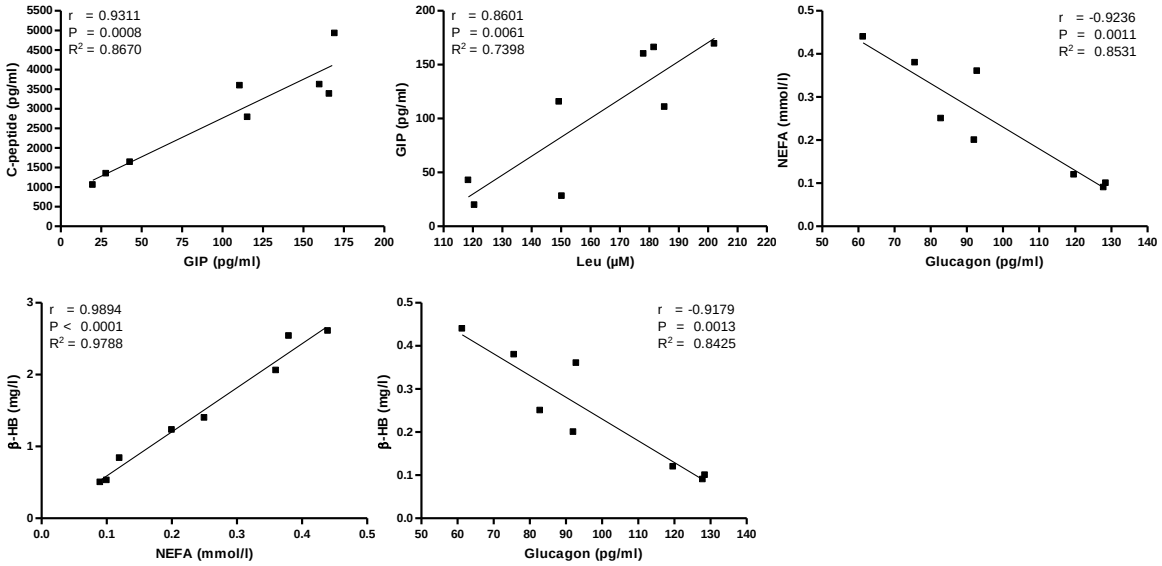


Figure 46: Correlations after intake of the GMP drink in the healthy group

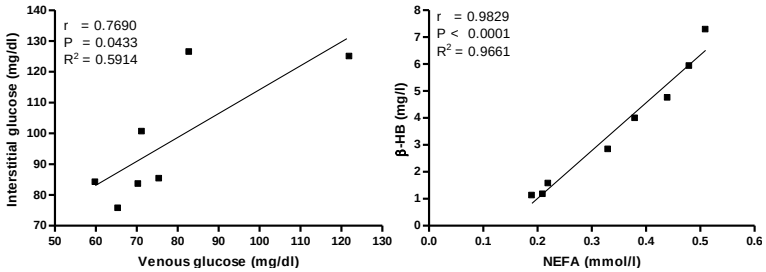
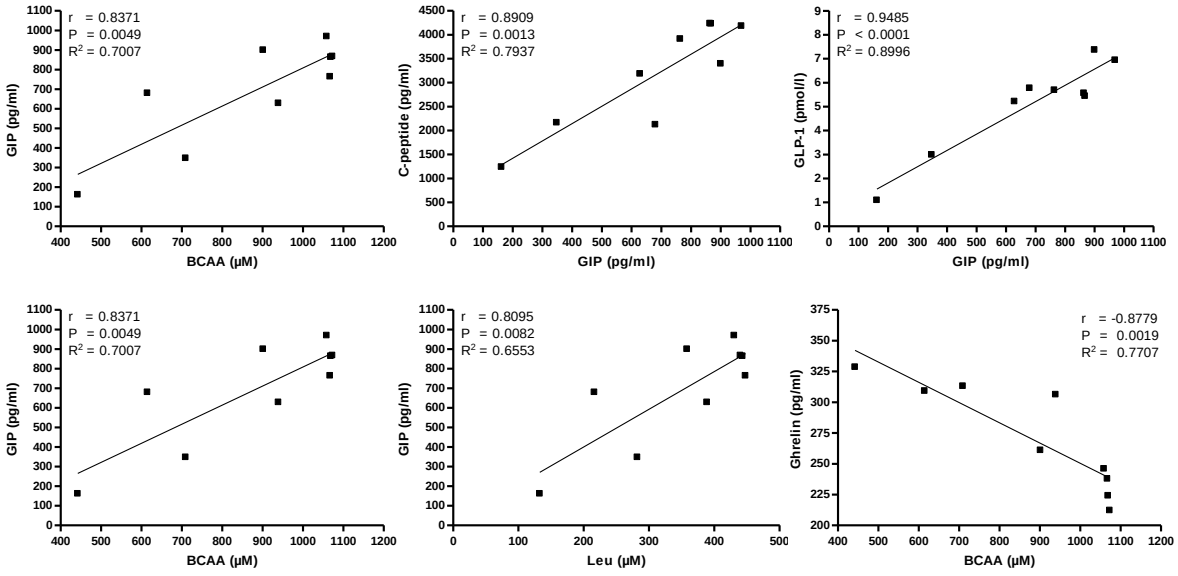


Figure 47: Correlations after intake of the MD19 drink in the healthy group



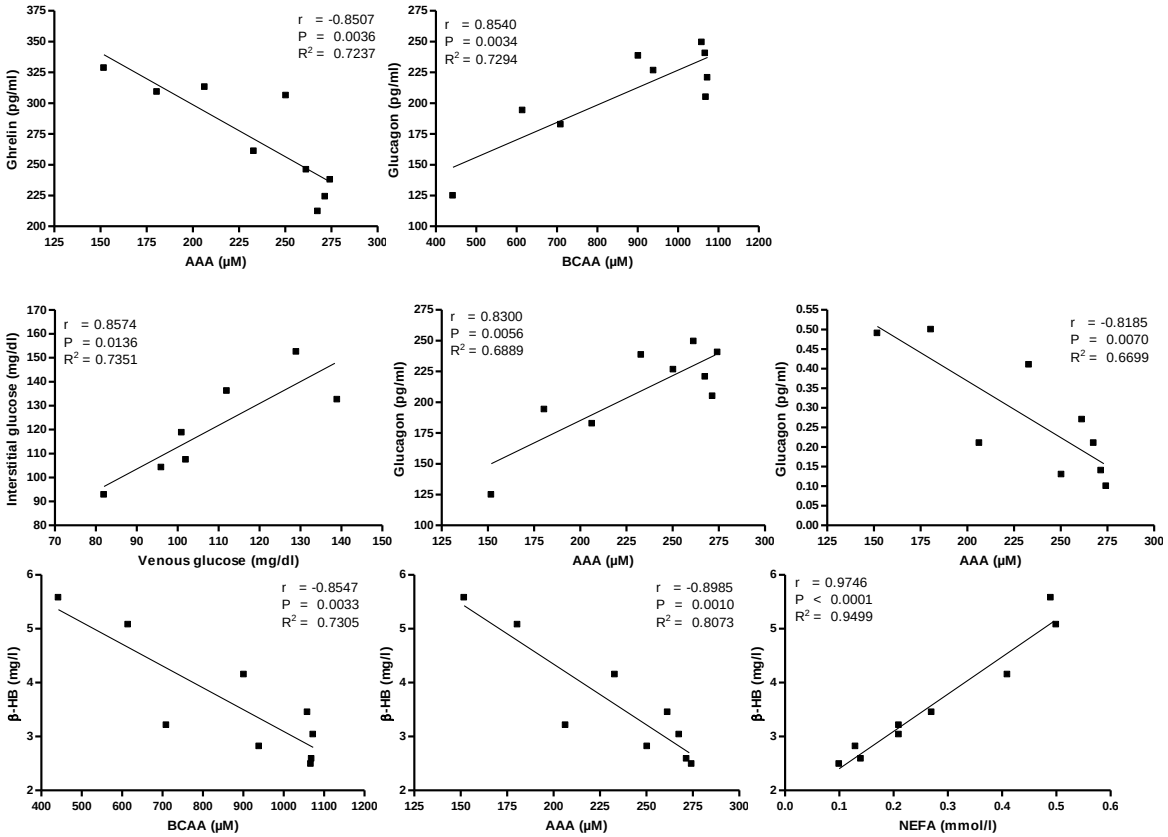


Figure 48: Correlations after intake of the WPI drink in the prediabetic group

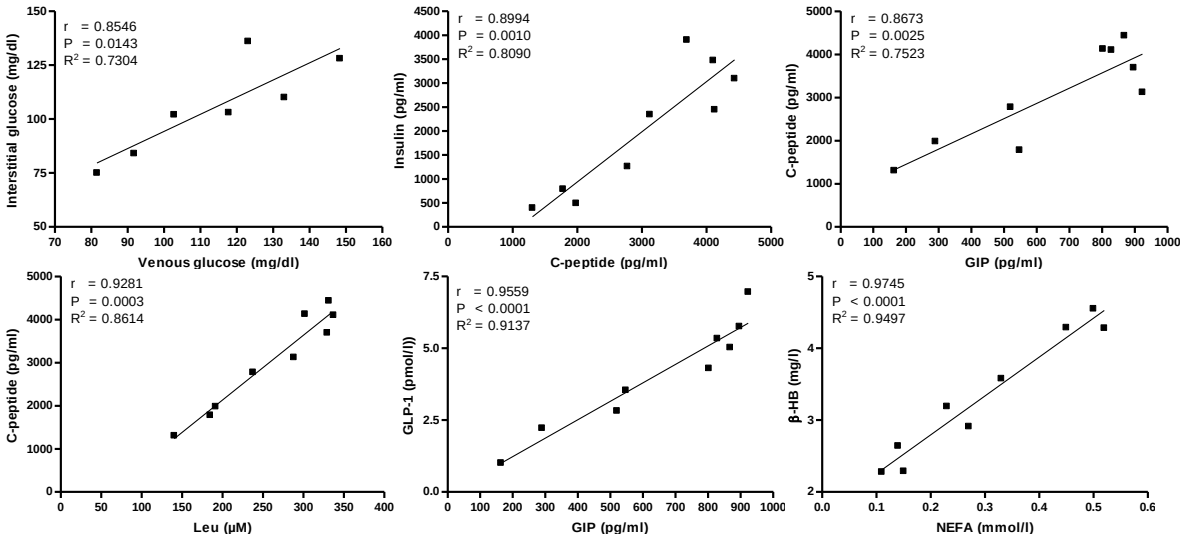


Figure 49: Correlations after intake of the CP drink in the prediabetic group

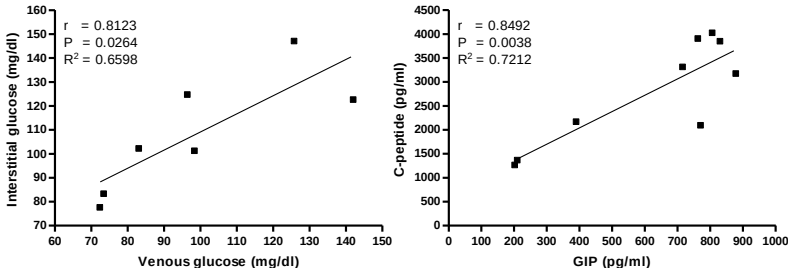


Figure 50: Correlations after intake of the GMP drink in the prediabetic group

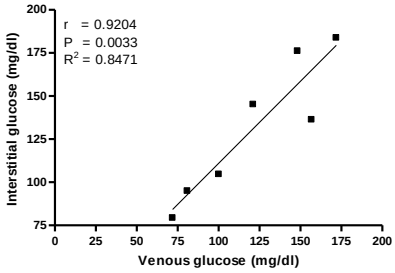


Figure 51: Correlations after intake of the MD19 drink in the prediabetic group