TECHNISCHE UNIVERSITÄT MÜNCHEN

Lehrstuhl für Ernährungsphysiologie

Metabolic interrelationships of amino acid metabolism in health and disease

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Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender:		Univ.-Prof. Dr. M. Klingenspor
Prüfer der Dissertation:	1.	Univ.-Prof. Dr. H. Daniel
	2.	Univ.-Prof. Dr. J. J. Hauner

Die Dissertation wurde am 26.10.2012 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 28.02.2013 angenommen.

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Summary

Metabolomics is a promising biomarker discovery tool. From a variety of human cohort studies applying different metabolomics techniques it becomes evident, that in particular amino acids and associated metabolites can serve as prognostic and/or diagnostic markers for diseases like insulin resistance and type 2 diabetes. However, intrinsic to those cohort studies is the limited availability of biosamples and almost all studies analyzed only one over-night fasting plasma sample. As metabolism is highly dynamic and little is known of the amino acid metabolome and its changes under various metabolic conditions, the subject of the present thesis was to assess which metabolic conditions cause major changes in the amino acid metabolome and to define its dynamics in a comprehensive phenotyping approach. The amino acid metabolome was thus analyzed in healthy volunteers submitted to different anabolic and catabolic challenges over a 4 days period with continuous blood and urine sampling. In order to assess the interplay between plasma (blood) and tissue metabolomes, mouse models were used to mimic the catabolic and anabolic state. To simulate the conditions of changes in the metabolome reported in human obesity and insulin resistance, high-fat feeding trials in mice were conducted.

Over the 4 days with various embedded challenges in human volunteers, the metabolome changed in a highly dynamic manner but appeared robust when reassessed in the same volunteer. In plasma, anabolic challenges such as an oral glucose or oral lipid tolerance test, and a physical activity test displayed metabotype specific changes over time, while extended fasting as catabolic condition failed to reveal that. Independent of any challenge, plasma and urinary 1-M-histidine concentrations declined over time and this suggested this metabolite as an exposure marker of previous meat intake.

Most prominent were consistent strong cross-correlations of branched-chain amino acids (BCAA) and α-aminobutyrate, which indicate that these amino acids underlay similar mechanisms of regulation. The opposed plasma responses to the anabolic and catabolic state in BCAA and α-aminobutyrate levels suggest them as potential metabolic markers. In response to the anabolic challenge oral glucose tolerance test the decline of the majority of plasma amino acids appear to be linked to insulin-mediated regulation of amino acid transporters removing these amino acids from

plasma. In an extended fasting state the “fingerprint” of metabolite changes was characterized by an increase of plasma BCAA and α-aminobutyrate levels, besides changes in ketone bodies, acylcarnitines, hepatic fatty acids and gluconeogenic compounds. This signature of the fasting metabolome was very similar to that described for the diabetic state suggesting that impaired insulin action causes a mild catabolic condition. Enhanced β-oxidation and thus elevated levels of non-esterified fatty acids in this state seems to inhibit the catabolism of BCAA. Despite the fact that the C57BL/6 mice submitted to high-fat feeding and obesity developed signs of the metabolic syndrome with hyperglycemia and hepatic triglyceride accumulation, plasma did not display the changes reported for the human condition such as increased BCAA concentrations. However, the high-fat feeding caused impairments of citrulline/arginine metabolism and amino acid profiling of different tissues (liver, skeletal muscle, small intestine and kidney) suggested that alterations in the hepatic metabolism might lead to the observed alterations.

Overall, systemic amino acid metabolism displays tight acute regulation mechanisms, yet, states on insulin resistance and diabetes cause significant alterations that are mimicked by the fasting condition in healthy volunteers and in mice. Extended fasting conditions might therefore be explored to investigate the causative mechanisms for amino acid changes that are frequently associated with the diabetic state.

Zusammenfassung

Umfassende Analysen von Stoffwechselprodukten („Metabolomics“) dienen u. a. der Identifizierung von Biomarkern. Vor allem Plasma-Aminosäuren und ihre Derivate haben sich als mögliche Biomarker einer Insulinresistenz und Diabetes mellitus Typ 2 in Kohortenstudien erwiesen. Meist basieren die Analysen nur auf einer Plasmaprobe im Nüchternzustand und berücksichtigen dabei nicht die Dynamik des Metaboloms. Das Ziel der vorliegenden Arbeit war deshalb, die Dynamik der Änderungen im Plasma- und Urinmetabolom mit dem Schwerpunkt auf den Aminosäuremetabolismus zu erfassen und deren zugrundeliegende Änderungen zu verstehen. Hierfür wurde in einer viertägigen Studie das Metabolom von gesunden Probanden in anabolen und katabolen Stoffwechsellagen unter Berücksichtigung zeitabhängiger Veränderungen untersucht. Der Schwerpunkt lag dabei auf der Analyse des Aminosäurestoffwechsels. Neben der Humanstudie, wurden die anabolen und katabolen Stoffwechselsituationen im Mausmodell simuliert. Des Weiteren, wurden mit Hilfe von Fütterungsstudien mit Hochfettdiäten an Mäusen die durch die Adipositas verursachten metabolischen Änderungen untersucht. Dabei wurde auch geprüft, inwieweit ein solches Mausmodell geeignet ist die Insulinresistenz bzw. einen diabetischen Zustand beim Menschen nachzustellen. In allen Mausstudien wurde die Analyse der Aminosäuren auf die Gewebe ausgeweitet.

Obgleich das basale Aminosäuremetabolom beständig war, unterlag es starken dynamischen und stoffwechselabhängigen Schwankungen. Anabole Interventionen und ein Ergometertest führten zu charakteristischen zeitlichen Veränderungen, die probandenspezifisch waren; nicht aber eine katabole Stoffwechsellage induziert durch fortgeschrittene Nahrungskarenz. Unabhängig von der Art der durchgeführten Interventionen, zeigte 1-Methylhistidin einen stetigen Abfall in den Plasma- und Urinspiegeln und dies lässt es als Indikator für einen vorangegangen Fleischkonsum erscheinen.

Gleichartige zeitliche Veränderungen der Spiegel der verzweigtkettigen Aminosäuren und von α-Aminobuttersäure deuteten auf gemeinsame Regulationsmechanismen hin, wobei sie durch ihre gegensätzliche Konzentrationsänderungen im Anabolismus und Katabolismus als Biomarker für eine anabole bzw. katabole Stoffwechsellage gelten können. Der nach einem Glukosetoleranztest bemerkenswerte Abfall der

Plasmakonzentrationen der Mehrheit der gemessenen Aminosäuren (unter anderem der verzweigkettige Aminosäuren und α-Aminobuttersäure) beruht vermutlich auf einer durch Insulin vermittelten Aktivierung von Aminosäuretransportern, die zur Elimination der Aminosäuren aus dem systemischen Kreislauf führt. Umgekehrt war der Anstieg des Plasmaspiegels der verzweigtkettigen Aminosäuren und α-Aminobuttersäure charakteristisch für eine durch Nahrungskarenz induzierte katabole Stoffwechsellage, welche zusätzlich mit Veränderungen der Plasmaspiegel von Ketonkörpern, Acylcarnitinen, glukogenen Metaboliten und Fettsäuren in der Leber einhergingen. Somit zeigt das Metabolom des Stoffwechselgesunden in der Nahrungskarenz nahezu dasselbe Muster wie dies von Übergewichtigen mit Insulinresistenz oder Typ 2 Diabetikern. Diese Ergebnisse lassen darauf schließen, dass bei erhöhter Rate der β-Oxidation, die mit einem Anstieg an freien Fettsäuren im Plasma einhergeht, eine Hemmung des Schlüsselenzyms des Abbaus der verzweigtkettigen Aminosäuren erfolgt, was zu einem systemischen Anstieg der verzweigtkettigen Aminosäuren und der α-Aminobuttersäure führt.
Eine Hochfettfütterungsstudie an C57BL/6 Mäusen, die zu Adipositas, Hyperglykämie und erhöhtem Leberfettgehalt – ähnlich dem metabolischen Syndrom beim Menschen – führte, zeigte dagegen kein Anstieg der verzweigtkettigen Aminosäuren im Plasma, wie er für diese Kondition beim Menschen mehrfach beschrieben wurde. Allerdings zeigte sich eine Beeinträchtigung des Citrulline/Arginine-Metabolismus. Die Analysen der Aminosäureprofile von Skelettmuskel, Leber, Dünndarm und Niere ließen darauf schließen, dass Veränderungen im Stoffwechsel der Leber zu den erhöhten Citrullinkonzentrationen im Plasma führten.

Zusammenfassend lässt sich feststellen, dass der Aminosäuremetabolismus strengen Regulationsmechanismen unterliegt, die sich mit charakteristischen Änderungen im anabolen und katabolen Stoffwechsel im Plasma zeigen. Der katabole Zustand in fortgeschrittener Nahrungskarenz führt im gesunden Probanden als auch im Mausmodell zu Änderungen im Plasma, die denen von Patienten mit Insulinresistenz und Typ 2 Diabetes gleichen. Damit könnte die Nahrungskarenz ein Model für die Untersuchung zugrundeliegender Mechanismen, die diese markanten Veränderungen im Aminosäuremetabolismus bedingen, sein.

1. Introduction

Each biological sample consists of a diverse and huge set of metabolites of different chemical properties. Approximately 8,000 metabolites are currently known to be found in humans (human metabolome database, version 2.5; see also [1]) and it is estimated that around 3,000 metabolites thereof are from endogenous origin [2]. Metabolomics is used as a term for the analysis of a wide range of metabolites and is the youngest of the "omics" technologies. While metabolomics is defined as "*the comprehensive analysis of the whole metabolome under a given set of conditions*" [3], the analysis focused on a specific class of compounds like amino acids, carbohydrates, or lipids is defined as metabolite profiling [3]. A huge spectrum of regulatory mechanisms concerning genes, transcripts and proteins affect metabolite concentrations as the final entity of each metabolic reaction. For instance, if patients are suffering from genetic disorders like phenylketonuria, maple syrup urine disease and medium-chain acyl-CoA dehydrogenase deficiency, altered metabolite concentrations in plasma can easily be detected [4]. In this regard, the newborn screening programs established in various countries and mostly employing tandem mass spectrometry (MS) techniques might be considered as the first metabolomics approaches.

In contrast to those inherited diseases, the most common non-communicable diseases such as diabetes, cancers or cardiovascular disorders have long time-lines for the development of pathologies. This means, that metabolism is slightly deregulated over a long time-period before disease becomes manifest. By using the advantages of gas chromatography (GC)-MS, liquid chromatography (LC)-MS or nuclear magnetic resonance (NMR) techniques, various studies have been conducted to find specific biomarkers for distinct diseases, for improved early diagnosis or for predicting disease progression. This covers colon [5] and kidney [6] cancers, Alzheimer disease [7] and impaired-glucose tolerance, type 1 and type 2 diabetes [8-14]. In case of insulin resistance and diabetes a variety of studies reported increased levels of plasma amino acids as "putative biomarkers" [8, 11, 12, 15]. In most of those cohort studies however only one fasting plasma sample (or 24 h urine sample) is collected on which analysis is based. Frequently neglected is the fact, that plasma or urinary concentrations of metabolites are subject to fast but also

sustained alterations. In this respect, it seems mandatory to define properly the “normal and healthy” metabolome, which is an ever-changing, dynamic entity.
Hence, the general aim of this study was to investigate the amino acid metabolism and its interrelationships to other compound groups under defined metabolic conditions. We used the approach to cause deflections by imposing defined anabolic and catabolic conditions in a highly standardized human intervention study and in mouse models. In all studies, MS based metabolite profiling techniques were applied.

1.1. Amino acids and their metabolism

1.1.1. Amino acids and proteins

Amino acids are classified as essential and non-essential ones and can additionally be grouped according to their side-chains (non-polar, polar, basic or acidic) (Table 1). The different side-chains are critical for the various functional roles within proteins [16]. Proteins are macromolecules that are built up from the 20 proteinogenic amino acids, can undergo posttranslational modifications and appear in a huge diversity in the cell. They serve as structural components and are involved in transport mechanisms, have immune functions, and are important in cell signaling processes. As enzymes, proteins play a pivotal role in the regulation of biochemical processes.
A 70 kg man has a protein amount of about 12 kg, while the free amino acid pool is only around 200 g [17]. Skeletal muscle accounts for approximately 60% of the whole-body protein mass [18] and for more than 25% of the whole-body protein turnover [19]. Under physiological conditions, there is a continuous and well-controlled balance between protein breakdown and protein *de novo* synthesis, and therefore exchanges between protein bound amino acids and free amino acids occur. Dietary amino acids and amino acids derived from protein breakdown either can be metabolized into products like polyamines, glutathione and neurotransmitters or be used as energy substrate for adenosine triphosphate (ATP) production. The main site of oxidation differs between the amino acids, as valine, leucine and isoleucine, asparagine, aspartate and glutamate are directly oxidized in skeletal muscle [17], while other amino acids are catabolized mainly in the liver. Except for the solely ketogenic compounds leucine and lysine, amino acids can serve as substrates for gluconeogenesis (see also Table 1).

Table 1: Classification of the 20 proteinogenic amino acids
Amino acids are categorized according to the chemical properties of the side-chain. They are further grouped into non-essential and essential amino acids and specified as glucogenic, ketogenic or both glucogenic and ketogenic.

	Amino Acid	3-letter code	1-letter code	Essential
Unpolar	Glycine[a]	Gly	G	
	Alanine[a]	Ala	A	
	Proline[a]	Pro	P	
	Methionine[a]	Met	M	x
	Cysteine[a]	Cys	C	
	Phenylalanine[c]	Phe	F	x
	Tryptophan[c]	Trp	W	x
	Valine[a]	Val	V	x
	Leucine[b]	Leu	L	x
	Isoleucine[c]	Ile	I	x
Polar	Serine[a]	Ser	S	
	Threonine[c]	Thr	T	x
	Tyrosine[c]	Tyr	Y	
	Asparagine[a]	Aan	N	
	Glutamine[a]	Gln	Q	
Basic	Lysine[b]	Lys	K	x
	Arginine*[a]	Arg	R	x
	Histidine[a]	His	H	x
Acidic	Aspartic acid[a]	Asp	D	
	Glutamic acid[a]	Glu	E	

* Arginine is essential in young mammals [20, 21].
[a] glucogenic amino acids
[b] ketogenic amino acids
[c] both glucogenic and ketogenic amino acids

1.1.2. Regulation of the amino acid and protein metabolism

Several hormones influence protein metabolism and hence affect the amino acid handling and distribution. The anabolic hormone insulin reduces protein breakdown [22] and increases protein synthesis [23]. In addition, insulin enhances amino acid transport into skeletal muscle cells [24, 25]. Antagonistic to insulin are glucagon, catecholamines (epinephrine and nor-epinephrine) and glucocorticoids. Glucagon plays a pivotal role for hepatic amino acid uptake and increases glucose production via gluconeogenesis [26]. Furthermore, glucagon stimulates the amino acid disposal after an amino acid load which subsequently reduces the amino acid availability and therefore protein synthesis rate [27]. Increased plasma concentrations of the glucocorticoid cortisol enhance the appearance rate of labeled leucine and phenylalanine which suggests an increase in protein breakdown [28].

There is evidence that amino acids itself play a role in metabolic regulation. It has been demonstrated that alanine triggers insulin secretion in a cell culture system of beta-cells [29], with similar findings for leucine [30, 31] and glutamine. Particularly glutamine seems to enhance leucine stimulated insulin secretion [31]. Furthermore, amino acids play a role in protein turnover, as they are able to activate the

mammalian target of rapamycin (mTOR). mTOR is located in the cytoplasm and part of two functional complexes mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is sensitive to nutrients and a key regulator of translation, transcription and the control of autophagy (for review [32]). There is evidence that amino acids mediate the activation of mTORC1 via class 3 phosphatidylinositol 3 (PI3)-kinase (also known as human vacuolar protein sorting 34: hVps34) and further stimulates p70 S6 kinase 1 (S6K1) [33, 34]. In a negative feedback loop, S6K1 reduces the insulin receptor substrate 1 mediated activation of PI3-kinase [35] and thus blunts insulin action. This is in line with the report of Baum and coworkers showing in rats, that oral administration of leucine reduces the duration of insulin induced activation of PI3-kinase [36]. The current model of amino acid mediated activation of mTORC1 is displayed in Figure 1.

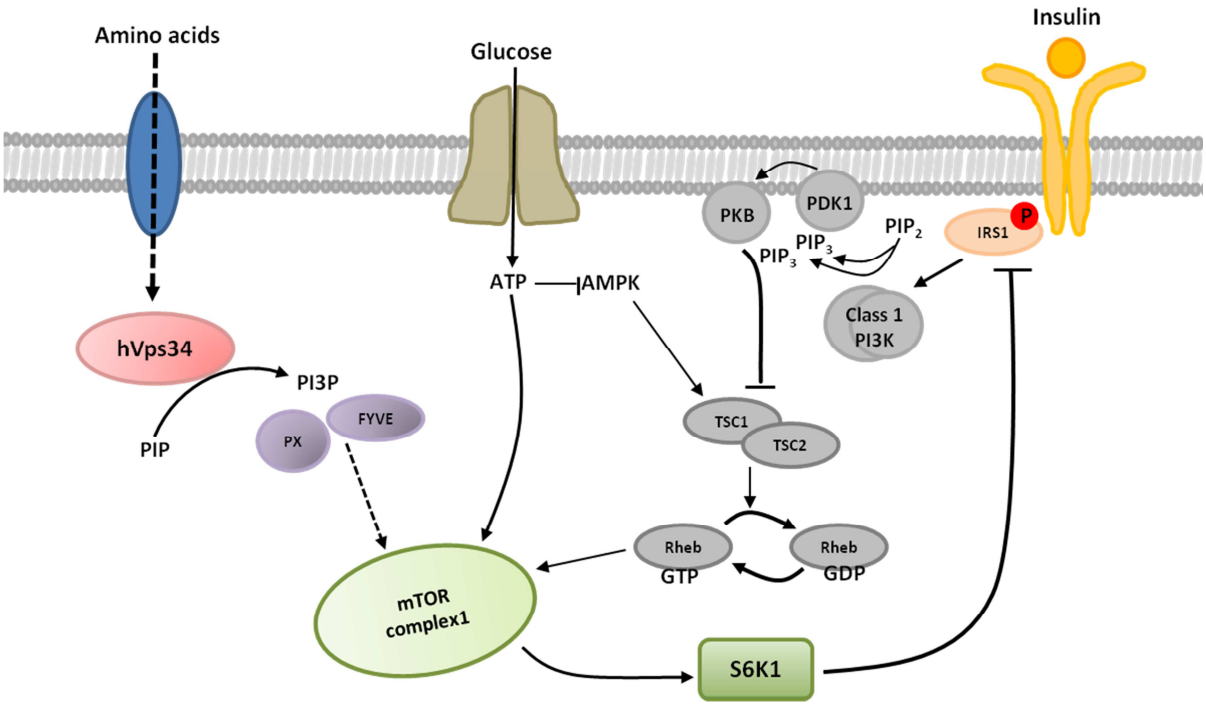


Figure 1: The signaling pathway of mTOR complex 1 and its interaction with the insulin signaling cascade.

Amino acids stimulate hVps34 activity. hVps34 is proposed to require PI3P, which than recruits proteins containing FYVE or PX domains leading to the activation of the mTOR complex 1. Further, increase of the cellular ATP concentration due to glucose uptake activates mTOR complex 1. Insulin binding to the insulin receptor leads to enhanced tyrosine phosphorylation of IRS1 and thus activating mTOR complex 1. Downstream target of activated mTOR complex 1 is S6K1 being phosphorylated. S6K1 diminishes the insulin signaling mediated by IRS1 by a negative feedback loop. For reasons of completeness and to provide an detailed overview, grey items are also drawn. (adapted from [32, 37, 38])

Abbreviations: hVps34, human vacuolar protein sorting 34; PIP, phosphatidylinositol phosphate; PI3P, phosphatidylinositol 3-phosphate; PX, phosphoinositide-binding structural domain; FYVE, **F**ab1p, **Y**OTB, **V**ac1p, **E**EA1, zinc finger domain; mTOR, mammalian target of rapamycin; S6K1, p70 S6 kinase 1; Rheb, ras homolog enriched in brain; TSC1, tuberous sclerosis protein 1; TSC2, tuberous sclerosis protein 2; PKB, protein kinase B, PDK1, posphoinositide-dependent kinase; PIP_2, phosphatidylinositol (4,5)-bisphosphate; PIP_3, phosphatidylinositol (3,4,5)-triphosphate; class 1 PI3K, class 1 phosphatidylinositol 3-kinase; IRS1, insulin receptor substrate 1.

Taken together, the metabolism of amino acids and proteins is controlled at various levels by several hormones and other regulators. Additionally, amino acids themselves seem to provide an input to cellular signaling processes via mTORC1.

1.1.3. Metabolism of citrulline

As it will be described later, the metabolism of the non-proteinogenic amino acid citrulline has some unique features and is therefore addressed here with some introductory remarks. Citrulline metabolism spans across small intestine, liver and kidney (Figure 2).

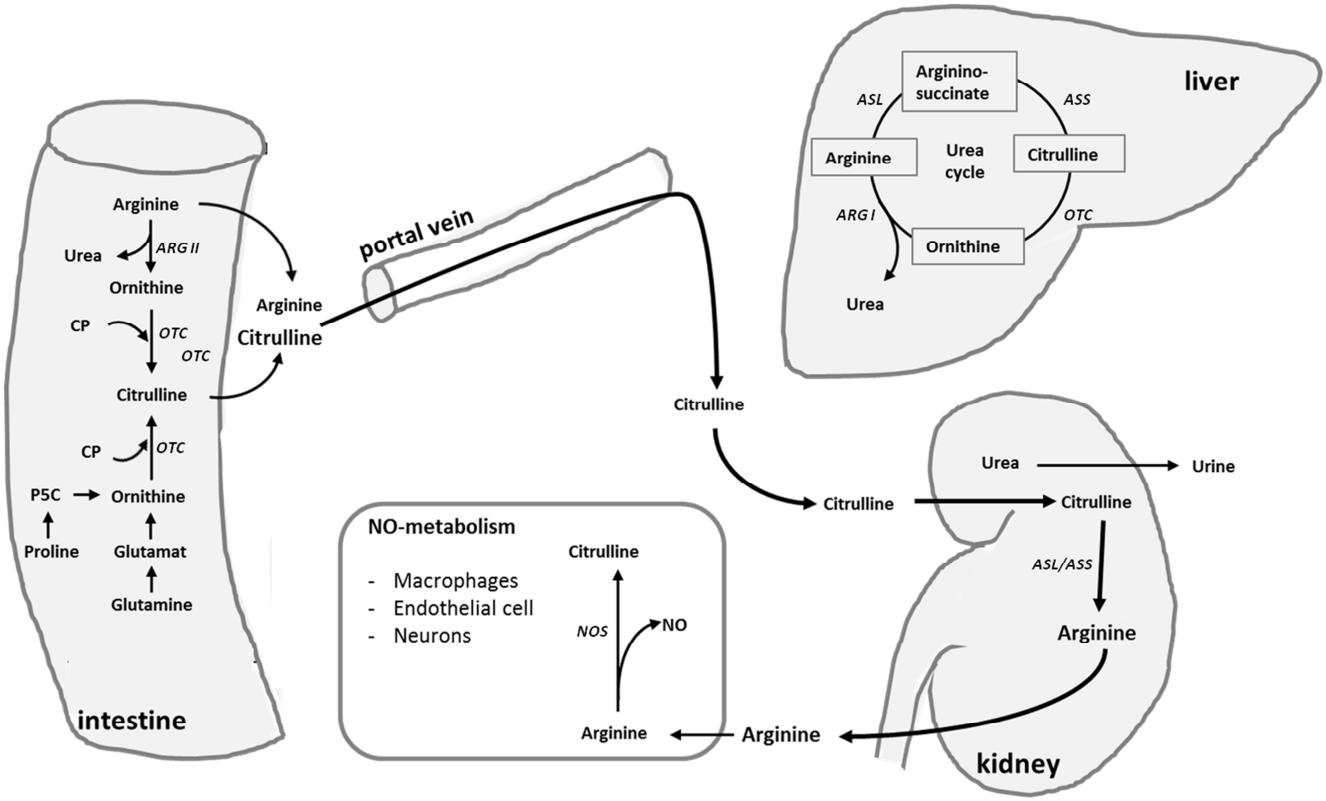


Figure 2: The interorgan metabolism of arginine and citrulline
Abbreviations: Arg I, arginase I; Arg II, arginase II; ASL, arginino succinate-lyase ; ASS, arginino succinate-synthetase; CP, carbamoyl-phosphate; OTC, ornithine carbamoyl transferase; P5C, pyrroline-5-carboxylate; NO: nitric oxide; NOS, nitric oxide synthase; (adapted from [39-41]).

Citrulline is produced in and released from the intestine. There is still a controversial discussion about the main precursor of citrulline. For humans, glutamine is considered as main precursor [42], whereas in rodents, it was shown that mainly arginine is converted to citrulline in the enterocyte [43, 44] but that glutamine favors

the production of citrulline in rats [44]. Loss of intestinal arginase II in mice leads to a reduction of intestinal citrulline release [45], further supporting the notion that dietary arginine is the main precursor of citrulline synthesis in mice [46].

In liver, citrulline is an intermediate of the urea cycle and thus involved in nitrogen detoxification. There are species specific differences described to which extend liver is able to take up or release citrulline from or into circulation [42, 47, 48]. However, citrulline is still considered to mask arginine by bypassing the liver [47]. Arginine is metabolized by the liver to a large extend [49] and the transformation of arginine to citrulline in the intestine is believed to prevent arginine extraction by liver. Finally, citrulline is taken up by the kidney and reconverted to arginine for systemic delivery [42, 47, 48]. Thus, the intestinal-renal citrulline cycle ensures arginine supply for the periphery. Additionally, citrulline is synthesized from arginine as a byproduct of the nitric oxide pathway.

1.1.4. Metabolism of branched-chain amino acids

The aliphatic amino acids valine, leucine, and isoleucine have a non-linear side chain (Figure 3) and are therefore grouped and collectively defined as branched-chain amino acids (BCAA).

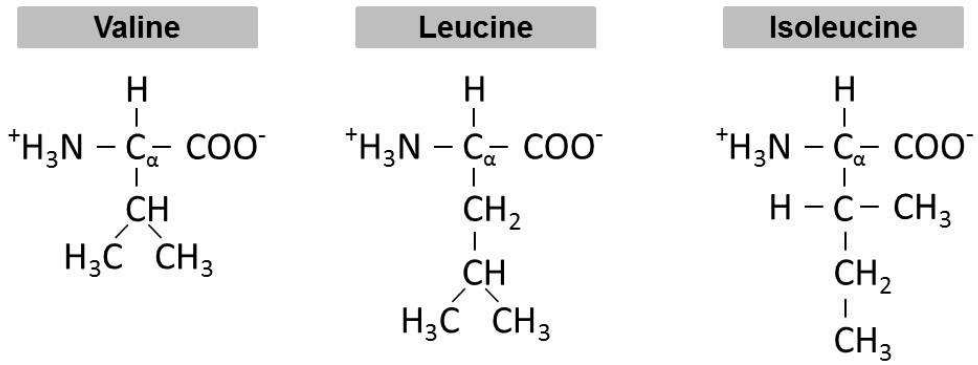


Figure 3: Structure of valine, leucine and isoleucine

As early as in the 1970s, it was reported that plasma BCAA concentrations rise after meat consumption disproportional [50, 51]. BCAA account for approximately 15% to 25% of the total protein intake [52], but in contrast to other amino acids, BCAA are not degraded in liver. Thus, dietary intake of BCAA directly influences the plasma concentrations [52]. In muscle tissue, BCAA are metabolized primarily and account

for about 35% of the total essential amino acids [53]. All three BCAA share a common catabolic pathway (Figure 4).

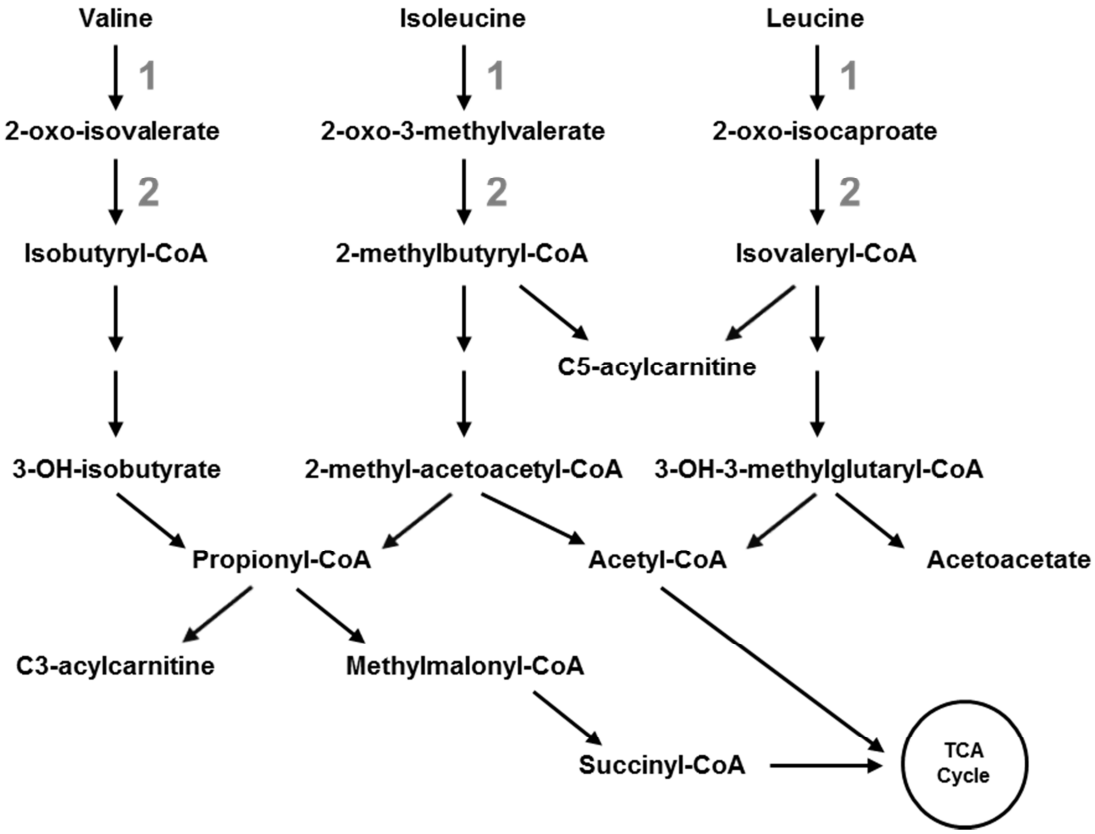


Figure 4: Scheme of BCAA catabolism
1. branched-chain amino acid aminotransferase, 2, branched-chain α-keto acid dehydrogenase complex. (adapted from [54, 55])

The branched-chain amino acid aminotransferase (BCAT) mediates the initial transamination step to form the respective branched-chain α-keto acids (BCKA). This reaction is reversible and α-ketoglutarate acts as acceptor of the amino group. The second and rate-limiting step is an irreversible oxidative decarboxylation catalyzed by the mitochondrial branched-chain α-keto acid dehydrogenase complex (BCKD) and commits the BCAA/BCKA to degradation. Leucine is a ketogenic amino acid and is ultimately oxidized to acetoacetate and acetyl-CoA, whereas valine is glucogenic and oxidized to succinyl-CoA. Isoleucine forms both, ketogenic and glucogenic products (acetyl-CoA and succinyl-CoA) [54].

1.1.5. Amino acid metabolism and insulin resistance

In 1968, Adibi reported that obese females displayed higher plasma BCAA concentrations than lean volunteers [56]. Higher plasma concentrations of BCAA were also found in type 2 diabetics and in poorly controlled type 1 diabetics [57-59]. This phenomenon has been rediscovered by metabolomics based biomarker discovery approaches. Several studies revealed that increased BCAA concentrations associate with obesity [15], insulin resistance and type 2 diabetes mellitus (T2DM) [8, 11, 60]. In a retrospective study, it could be established that the increases in plasma BCAA, tyrosine and phenylalanine levels are predictive for the risk of developing diabetes [12]. But not only these cohort studies have provided evidence for an association of alterations in amino acid levels in obesity, insulin resistance and diabetes, acute challenge tests also have demonstrated such a link. After an oral glucose tolerance test (OGTT), the insulin-dependent activation of amino acid transporters, which are needed for the removal of amino acids from plasma was blunted in individuals with impaired glucose tolerance [61]. As the BCAA and in particular leucine are considered as the agonist of the mTOR pathway, studies have also specifically addressed the role of BCAA in insulin resistance.

In rodent models with obesity supplementation trials with either BCAA or only leucine have produced controversial findings. In rats, BCAA administration reduced the weight gain in animals on high-fat diets, but revealed an impaired insulin sensitivity of organs [15]. Other studies with a leucine supplementation in drinking water revealed improved insulin signaling and diminished sizes of fat depots in mice [62, 63]. This improvement is opposed to the hypothesis that leucine can inhibit the insulin signaling cascade via stimulation of mTOR ([36], see 1.1.2).

In addition to this function of BCAA or leucine on cell signaling, metabolomics studies provide additional hints to impairments in metabolism in obesity or insulin resistant states. A recent paper by Fiehn *et al.* showed in diabetic women that increased plasma valine concentrations were inversely correlated to plasma propionyl-CoA and the authors proposed a link between BCAA catabolism and tricarboxylic acid (TCA) cycle activity [8]. A review by Adams summarized the current knowledge of BCAA catabolism in obesity, insulin resistance and T2DM. These states are characterized by high levels of free fatty acids, increased NADH/NAD^+ ratio and enhanced generation of acetyl-CoA and Adams hypothesized that this environment may lead to

inhibition of the BCKD, which would reduce BCAA oxidation. Degradation products of BCAA are important precursors of anaplerotic reactions for the TCA cycle and may thus affect the flow of substrates through the TCA cycle [64]. This postulated model of “anaplerotic stress” [8] that links BCAA catabolism and TCA cycle impairments of course needs experimental proof. Besides amino acids, metabolomics approaches have also identified plasma concentration changes of free fatty acids, various acylcarnitine species, and ketone bodies in subjects with impaired glucose handling and diabetic patients [8, 11, 60, 65]. Based on an untargeted profiling approach and statistical analysis using random forest classification, Gall and coworkers identified 2-OH-butyrate as a potential early biomarker for insulin resistance [60]. 2-OH-butyrate is a catabolite that can be derived from methionine and threonine metabolism [66] which further supports that insulin resistance impairs amino acid metabolism beyond the BCAA.

1.2. Metabolism and the need for constant energy supply

Proper cell function requires a continuous supply of energy substrates. External energy supply provided in form of carbohydrates, lipids and amino acids occurs via meals in which the organism has to shift macronutrient oxidation, disposal and storage. Liver, skeletal muscle and in particular adipose tissue are energy storage tissues. Time between meals or extended fasting periods require nutrient availability from these endogenous stores and a switch to utilize fatty acids as prime substrates for oxidation. These two major phases of energy metabolism are defined here as anabolic and catabolic states. Pathways involved in energy regulation mechanisms are predominantly controlled by the action of insulin and influence simultaneously glucose, amino acid and fatty acid metabolism.

1.2.1. The anabolic state

After food intake, there is a rise in blood glucose concentrations that together with intestinal hormones promotes insulin release from β-cells with a concomitant elevation of systemic insulin concentrations. Directly after food intake, glucose is the major energy source. Insulin increases the rate of glycolysis and suppresses endogenous glucose release from glycogenolysis and gluconeogenesis. As glycogen

storage capacity is limited compared to lipid storages, carbohydrates consumed in excess enter as acetyl-CoA *de novo* lipogenesis followed by esterification with glycerol to form triglycerides in liver and adipose tissue. Hepatic triglycerides are then incorporated into very low density lipoprotein particles and released into circulation for transport to the adipose tissue and storage [67]. Characteristic for the anabolic state is also a suppression of lipolysis by action of insulin and increased influx of glucose but also amino acids into tissues. As shown in humans, an insulin infusion causes a reduced amino acid release from forearm muscle [22] while protein ingestion promoted a net uptake of amino acids and mainly BCAA into forearm muscle up to 4 hours later [50]. Thus, amino acid breakdown and protein biosynthesis are affected by insulin and the presence of adequate amounts of free amino acids. Dietary amino acids per se also seem to play a pivotal role in hepatic protein synthesis in the postprandial state [68]. When free amino acids are administered by the portal vein protein synthesis in liver is increased [69]. In humans it was shown, that about 60% of ingested ^{15}N-labeled protein is extracted by the splanchnic bed and that 40% of the nitrogen provided is used for protein synthesis [70].

Recent studies also assessed the metabolite response to an OGTT in healthy volunteers by applying metabolomics technologies [10, 71-73]. The OGTT is the simplest method to study the condition of an anabolic state and is highly standardized [74]. Spégel *et al.* used a GC-MS based approach and measured the metabolic response of about 200 metabolites in plasma. Fatty acids and several amino acids including the non-proteinogenic amino acid ornithine decreased after glucose ingestion [72]. The reduction of various plasma amino acids after an OGTT, was also shown by Skurk *et al.* [73]. Based on a bioinformatical approach it was suggested, that the metabolic response to glucose administration may involve the action of distinct solute carrier proteins in organs such as muscle and liver for the removal of the amino acids from plasma and this may depend on the insulin sensitivity in the subjects [61]. Moreover, changes in metabolites which were not considered previously as regulated after glucose ingestion included bile acids, urea cycle intermediates, carnitines and lysophosphatidylcholines [10, 71]. Of note, metabolite responses showed different patterns. For instance, while bile acid species displayed increases in plasma and a biphasic kinetic behaviour, C10:0 and C12:0 acylcarnitines decreased and lysophosphatidylcholine levels increased [71]. Thus,

the anabolic response to a simple OGTT is far more complex than previously considered and metabolomic technologies offer more insights in the organisms' adaptation to a glucose load. However, with an OGTT only glucose is delivered and this of course does not reflect the complex nutrient pattern provided by a meal.

1.2.2. The catabolic state

Starvation

In the catabolic state, when blood insulin concentration is low and the concentration of glucagon rises [75], endogenous energy storage pools are utilized to match energy requirements. Figure 5 gives a simplified schematic overview of metabolic processes that take place during such a state.

A comprehensive review about fuel utilization under starvation conditions was provided by Cahill [76]. During an initial fasting period (over-night fast), glycogen ensures the maintenance of normal blood glucose levels and only about 20% to 35% of the hepatic glucose release is provided by gluconeogenesis in healthy as well as diabetic subjects [58, 77]. However, glycogen stores are completely depleted after 48 hours [76] and the percentage of glucose provided by gluconeogenesis increases progressively. Especially muscle proteolysis supplies substrates for hepatic glucose production. Fryburg *et al.* showed that muscle proteolysis is increased – determined by the release of [^{3}H]phenylalanine and [^{14}C]leucine from forearm - by 116% and 233% respectively after 60 hours of fasting as compared to an over-night fast [22]. Additional substrates for *de novo* glucose production are lactate and pyruvate (cori cycle) and glycerol from lipolysis [76]. There is also a flux of non-esterified fatty acids (NEFA) from adipose tissue to skeletal muscle for β-oxidation and to liver for ketoneogenesis. Especially during extended fasting periods and in starvation hepatic ketone body production becomes important. After 3 days of fasting, endogenous glucose production decreases [75] due to diminished glucose requirements. Although cells exclusively dependent on glucose (i.e. bone narrow or red blood cells) are served by gluconeogenesis, brain metabolism adapts to utilize ketone acids rather than glucose [76]. Thus, in starvation, the oxidation of ketone bodies preserves body protein stores as well as functional proteins to ensure proper cell functions like transport, immune response, cell signaling and enzyme activity. Changes in plasma

amino acids in response to food deprivation however are not uniform. Already in 1969, Felig *et al*. demonstrated in starved obese subjects over 40 days that there is a group of amino acids (BCAA, α-aminobutyrate and methionine) that display an early increase in plasma concentrations, while other amino acids increased (glycine, serine, threonine) time-delayed after day 5 or decreased in concentrations (i.e. proline, alanine and tryptophan) [78].

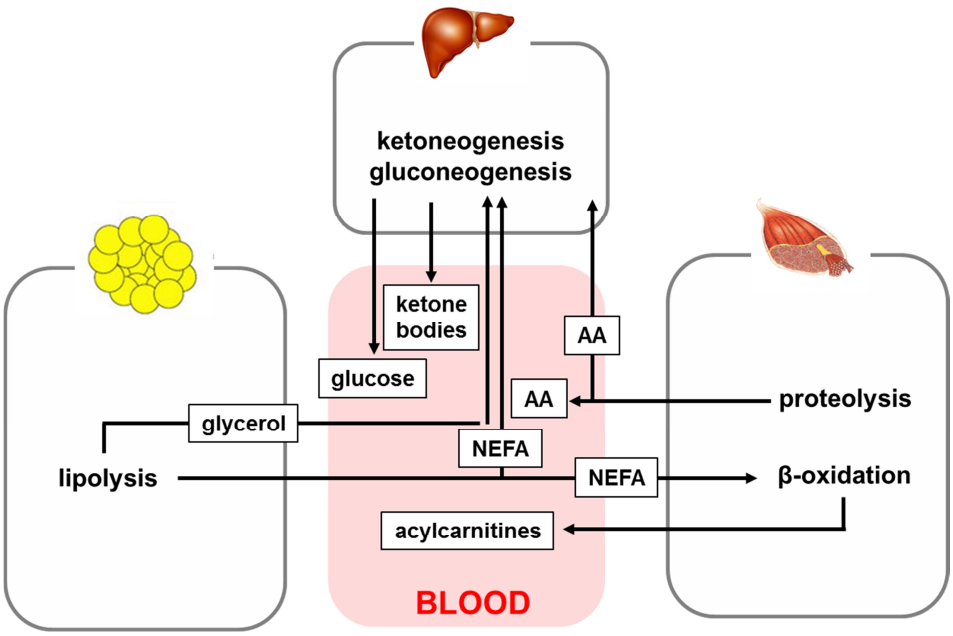


Figure 5: Schematic overview of metabolic processes in the catabolic state involving adipose tissue, liver and skeletal muscle
Abbreviations: AA, amino acids; NEFA, non-esterified fatty acids.

Although various metabolomics studies have profiled plasma or urine samples from humans after an over-night fast [10, 13, 60, 79-81], comparable studies assessing the response caused by extended fasting are limited [82]. That is one of the conditions targeted with the present project. Therefore, we here define the over-night fasting sample (plasma/urine) as the basal fasting sample. An extended fasting sample is defined as a fasting period of about 36 hours and starvation is defined as a period of food deprivation for more than 48 hours.

Physical activity

Physical activity is defined as "*any bodily movement produced by skeletal muscles that results in energy expenditure*" [83]. During physical activity, there is an enhanced demand for ATP in skeletal muscle, mostly in a catabolic condition. In the early 1970s, Felig and coworkers studied amino acid metabolism during physical activity [84, 85]. They observed a net flux of alanine from skeletal muscle to the hepatic tissue for gluconeogenesis, which increases with exercise intensity and is correlated to arterial pyruvate [84] supporting the previously postulated glucose-alanine cycle [86] that was later also confirmed by Poortmans *et al.* in 1974 [87]. The glucose-alanine cycle (Figure 6) describes the exchange of hepatic glucose and skeletal muscle-derived alanine.

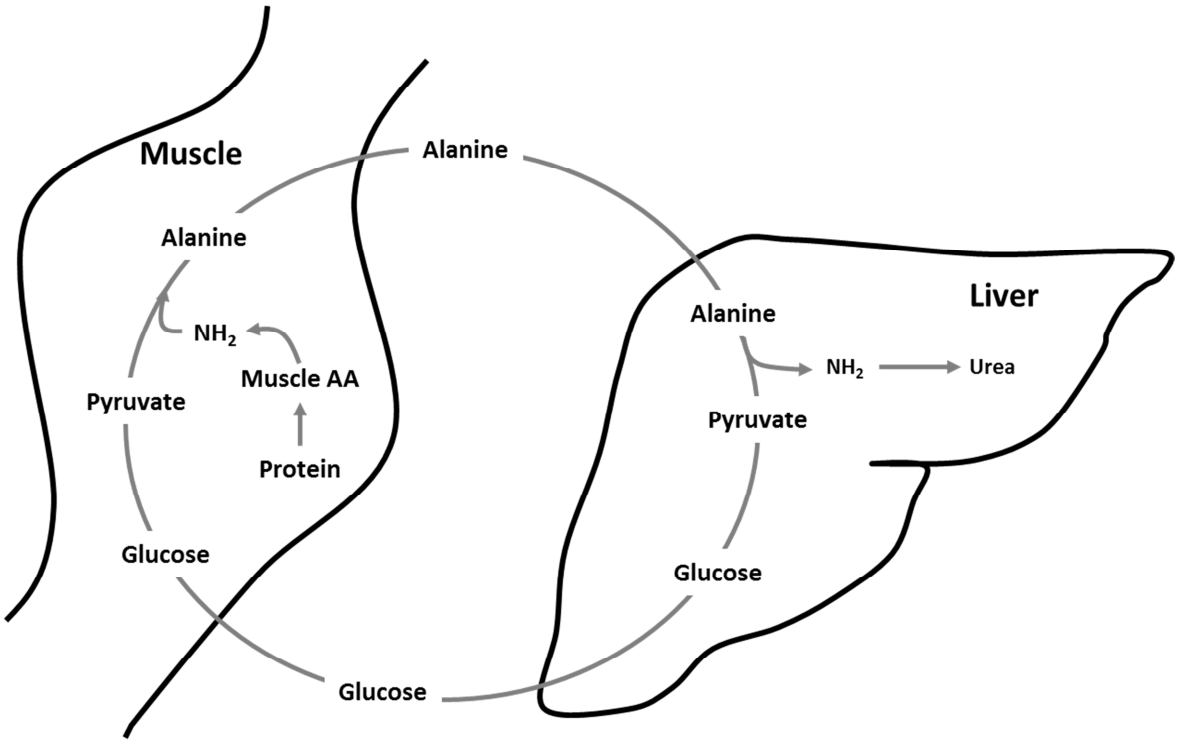


Figure 6: The glucose-alanine cycle
The glucose-alanine cycle describes the exchange of skeletal muscle alanine and hepatic glucose. Alanine transports nitrogen in form of NH2 to liver, where nitrogen detoxification via the urea cycle takes place. The carbon backbone in form of pyruvate is substrate for gluconeogenesis. The glucose produced is transported to the muscle and serves as energy substrate. The glycolysis product pyruvate is further transaminated to finally regain alanine (adapted from [86]].

Blood glucose and insulin levels decline during prolonged exercise, while glucose uptake into the muscle is upregulated throughout exercise [88]. In addition, fatty acid uptake and their utilization as energy source becomes more important with prolonged exercise [85, 88].

Based on LC-MS approaches, plasma metabolite changes induced by treadmill and cycle ergometer exercise have been studied [89]. Both physical activities induced similar changes in plasma metabolites reflected by increased glycolysis with elevations of pyruvate and lactate, enhanced lipolysis with increases of glycerol and in addition an increased release of amino acids (alanine and glutamine) mainly from muscle. Metabolites of the adenine nucleotide catabolism and of the TCA cycle were altered as well [89]. Directly after a marathon as well as 24 hours later, finishers displayed decreased plasma amino acid concentrations [90], whereas ketone bodies were increased [89]. Endurance exercise thus leads to similar metabolic adaptation phenomena as starvation, but the magnitude as well as the pattern of metabolite changes depends on intensity and duration of physical activity.

1.2.3. The metabolic flexibility concept

The reciprocal control of glucose (anabolic) and free fatty acid (catabolic) utilization for the bodies energy demands is according to Randle the “glucose-fatty acid cycle” [91]. The individual’s capability to change energy fuel selection has been described [92, 93] and the term “metabolic flexibility” was introduced and defined as “*the capacity to utilize lipid and carbohydrate fuels and to transit between them*” [94, 95]. Since this fuel selection is mainly controlled by insulin, insulin sensitivity becomes a critical determinant and consequently obese and type 2 diabetes patients have an impaired capability to switch between fatty acid and glucose oxidation. Obese volunteers were shown to possess a higher glucose utilization during fasting conditions accompanied by a reduced rate of fatty acid oxidation [96]. This pattern of energy expenditure remains unchanged during insulin stimulation whereas lean volunteers respond to insulin and display a higher rate of glucose oxidation [96]. The metabolic flexibility seems to be mirrored in changes in other metabolites concentration. In type 2 diabetics, insulin infusion induced a blunted reduction in plasma acylcarnitine levels compared to lean volunteers [65]. Furthermore, during

physical activity, trained individuals display a higher increase of plasma glycerol levels, which is a product of increased lipolysis, and higher levels of TCA products (fumarate, malate, succinate) [89] whereas the concentrations of glutamine and methionine in plasma increased more in non-trained individuals than in trained individuals. This suggests a higher rate of protein degradation in less trained volunteers [89]. Furthermore, the increase in TCA products also suggests a better anaplerotic support of the TCA cycle in trained individuals as compared to untrained people. This would be in line with the model termed “anaplerotic stress” [8]. Metabolic flexibility was also applied to conditions of a lipid challenge. Metabolite profiling in humans after an oral lipid load reveals a clear separation of individuals and this may reflect different ability to deal with the fuel supply. The same volunteers however could not be separated based on metabolite profiles in the fasting state [97]. Challenge tests therefore seem particularly suitable to reveal phenotypic differences between individuals.

1.3. Aim of work

This work focused on the human and murine metabolome under special consideration of amino acids since amino acids are described as potential markers for obesity and T2DM.

The “healthy” amino acid metabolome was investigated within the Human Metabolome (HuMet)-study. Male volunteers were challenged by different metabolic interventions, meaning a 36 hour fasting period, an oral glucose tolerance test (OGTT), a cycling ergometer test (CET), a standard liquid diet (SLD) test and an oral lipid tolerance test (OLTT). During the challenges, plasma and urine samples were collected for analysis of metabolite concentration changes in a time-resolved manner. As addressed before, special emphasis was given to the changes induced by the catabolic state represented by extended fasting and the anabolic condition as induced by an OGTT. The same conditions were studied in mouse models, which allowed to extend the analysis also to tissues such as skeletal muscle and liver. In view of the observations in obese humans and diabetics, the amino acid metabolome of plasma and tissues (liver, skeletal muscle, kidney and small intestine) was also profiled in C57BL/6 mice made obese by 12 weeks of high-fat (HF) feeding.

The overall purpose of the present study was to assess the dynamics of changes in the amino acid metabolome under defined metabolic conditions and to gain insights what might cause the changes in the diabetic state.

2. Results

2.1. Challenging the “healthy” amino acid metabolome

2.1.1. HuMet-Study

To assess the ‘healthy’ amino acid response to specific and defined metabolic conditions, we challenged 15 male healthy volunteers with a narrow body-mass index (BMI of 23.1 ± 1.76 kg/m²) and age range (27.8 ± 2.98 years) in a standardized manner [98]. Within four days volunteers underwent five metabolic challenges like fasting, an OGTT, a SLD, a CET and an OLTT (for study design see 4.3.2. and Figure 36). In total, we analyzed 50 plasma and 15 urine samples per volunteer. By using the iTRAQ-labeling approach combined with liquid chromatography tandem mass spectrometry (LC-MS/MS) technique, we were able to detect and quantify 28 and 32 amino acids and derivatives in plasma and urine, respectively.

Plasma amino acid levels after an over-night fasting period revealed a reproducible amino acid metabolome

Plasma samples after an over-night fasting period of 12 hours were measured at day 1, 3 and 4 of the study. Glutamine and alanine followed by valine and glycine displayed highest mean plasma concentrations (glutamine: 670.89 ± 87.39 µmol/l, alanine: 387.84 ± 80.79 µmol/l, valine: 278.07 ± 27.30 µmol/l and glycine: 253.53 ± 34.96 µmol/l). Amongst the amino acid with lowest concentrations were the derivatives P-ethanolamine, sarcosine and 3-M-histidine (P-ethanolamine: 3.31 ± 0.85 µmol/l, sarcosine: 4.54 ± 0.89 µmol/l and 3-M-histidine: 5.46 ± 1.27 µmol/l). In order to assess if the amino acid metabolome is a tightly regulated and thus robust entity, we performed repeated-measurement ANOVA for the three over-night fasting samples. Significant concentration changes were determined for ornithine, phenylalanine and the two amino acid derivatives 1-M-histidine and hydroxyproline at day 4 compared to day 1 and day 3 (Table 2). Nevertheless, 86% of the quantified plasma amino acids did not show any significant alterations when baseline samples were compared.

Table 2: Plasma amino acids concentrations of significantly changed amino acids after three over-night fasting periods

	Basal concentration (µmol/l)			
Amino acid	**Day 1**	**Day 3**	**Day 4**	***p*-value adj.**
Hydroxyproline	11.01 ± 2.42	11.83 ± 4.14	7.74 ± 1.16	0.003 [a,b]
Ornithine	55.13 ± 9.15	60.18 ± 11.12	46.51 ± 5.49	0.002 [a,b]
Phenylalanine	60.26 ± 7.20	58.64 ± 5.34	68.42 ± 6.78	<0.001 [a,b]
1-M-histidine	15.85 ± 3.71	15.50 ± 4.34	6.30 ± 1.83	<0.001 [a,b]

Data are presented as mean ± SD. *P*-value obtained by repeated-measurement ANOVA and adjusted with Benjamini-Hochberg correction. [a]$p < 0.05$ obtained by pair-wise comparison using Tukey post-hoc analysis when comparing day 1 and day 3. [b]$p < 0.05$ obtained by pair-wise comparison using Tukey post-hoc analysis when comparing day 1 and day 4.
Abbreviations: 1-M-histidine, 1-methyl-histidine.

Furthermore, ANOVA analysis was performed to determine within- and between subject variability of the three baseline samples. Eighteen of the 28 determined plasma amino acids and derivatives displayed subject variability (Suppl. Figure 38A). 3-M-histidine, alanine and serine displayed highest between-subject variability and the derivate P-ethanolamine displayed the highest within-subject variability.

Urine amino acid data was standardized with osmolarity

During the study, 15 spot urine samples were collected. Differences in collection time and embedded challenges influenced the urinary metabolome and normalization was required for the entire urinary data set. Creatinine, osmolarity and urine volume are usual approaches to normalize urinary metabolite concentrations [99]. Creatinine concentrations and osmolarity determined for each of the 15 x 15 urine samples displayed a high cross correlation ($r = 0.81$, $p < 0.001$). Yet, normalization of the amino acid data to osmolarity led to improved data validity. For instance, as displayed in Figure 7, tryptophan and ornithine were more homogenously distributed, when concentrations had been normalized to osmolarity compared to creatinine-normalized concentrations. In addition, data normalization with osmolarity strengthened the time-dependent changes that were observed for some amino acids, as for instance it is shown for valine (Figure 7). Furthermore, osmolarity-normalized data had a lower coefficient of variation (CV) and calculated median, minima and maxima of CV was lower compared to creatinine-normalized urine data as well (creatinine: $CV_{min} = 25.13$, $CV_{median} = 38.61$, $CV_{max} = 92.18$; osmolarity: $CV_{min} = 15.84$, $CV_{median} = 31.34$, $CV_{max} = 44.76$). This most likely led to the observed improvements in the data set when data was normalized to osmolarity and consequently osmolarity-normalized urinary amino acid data was used for further analysis.

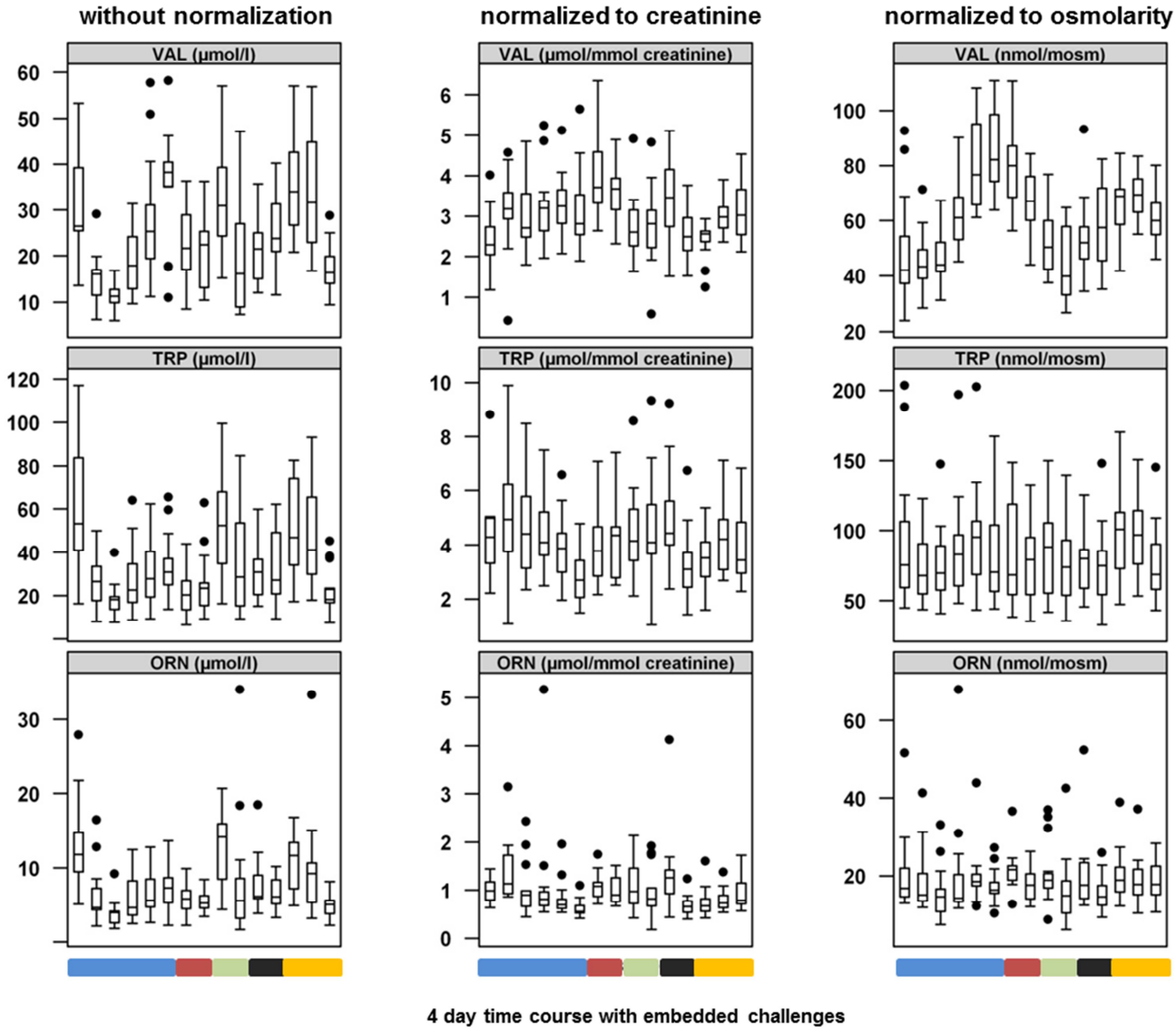


Figure 7: Selected urinary amino acid concentrations displayed without any normalization, normalized to creatinine and normalized to osmolarity for each collection time-point

Color-bars on the x-axes indicate the five metabolic challenges: Fasting, blue; SLD, red; OGTT, green; CET, black; OLTT, yellow. Black dots represent outliers.

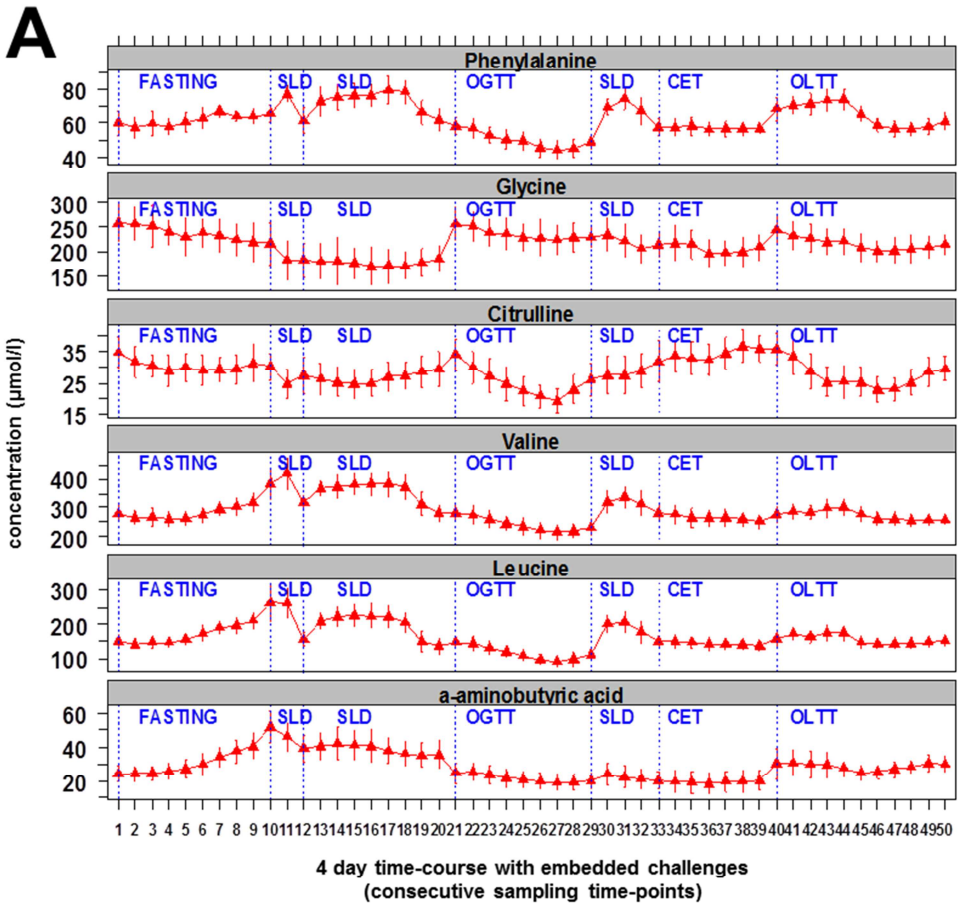
Abbreviations: ORN, ornithine; VAL, valine; TRP, tryptophan.

Repeated-measurement ANOVA of the first-void urine samples of day 1, day 3 and day 4 revealed, that 7 of the 32 (22%) detected amino acids displayed significant higher (arginine, argininosuccinate, glutamate, isoleucine, valine; $p < 0.05$) respectively lower concentrations (1-M-histidine, cystathionine; $p < 0.05$) on day 4 compared to day 1 and day 3. Like in plasma, urine phenylalanine concentrations were slightly higher at day 4 (day 1 = 69.99 ± 30.04 nmol/mosm, day 3 = 70.04 ± 21.39 nmol/mosm, day 4 = 86.74 ± 25.81 nmol/mosm, $p = 0.057$). We further

determined for the first-void urine samples subject variability. Compared to the analysis of plasma between- and within-subject variability, the CV in the urine amino acid data set was higher (Suppl. Figure 38B). Twenty-five of the 32 osmolarity-normalized amino acids displayed subject variability. Highest between-subject variability was found for lysine with a CV > 1.0, followed by β-aminoisobutyrate and cystathionine. Highest within-subject variability was found for β-aminoisobutyrate.

Some amino acids showed coherent dynamic changes in plasma independent of the challenges

In the entire study five metabolic challenges were embedded and concentration profiles of selected plasma amino acids are displayed in Figure 8A. In order to estimate challenge-independent associations of the amino acid time-profiles we applied correlation analysis for the whole plasma data set. As it is pictured in Figure 8B for a subset of amino acids, over the entire 4 days, highest cross-correlations were found between the BCAA valine, leucine and isoleucine (r = 0.92 between valine and leucine; r = 0.91 between valine and isoleucine; r = 0.95 between leucine and isoleucine). BCAA share identical chemical properties and biological pathways, as they an aliphatic side-chain, belong to the group of the essential amino acids and metabolism occurs via the same enzymes. Interestingly, as depicted in Figure 8B, the time-dependent changes of the derivative α-aminobutyrate and the aromatic amino acid phenylalanine were associated with the dynamic changes observed for the BCAA as well (range of Pearson correlation between r = 0.65 to r = 0.79). This indicates that independent of the challenge tests applied and thus independent of the metabolic state the BCAA, α-aminobutyrate and phenylalanine underlie similar regulation mechanisms.



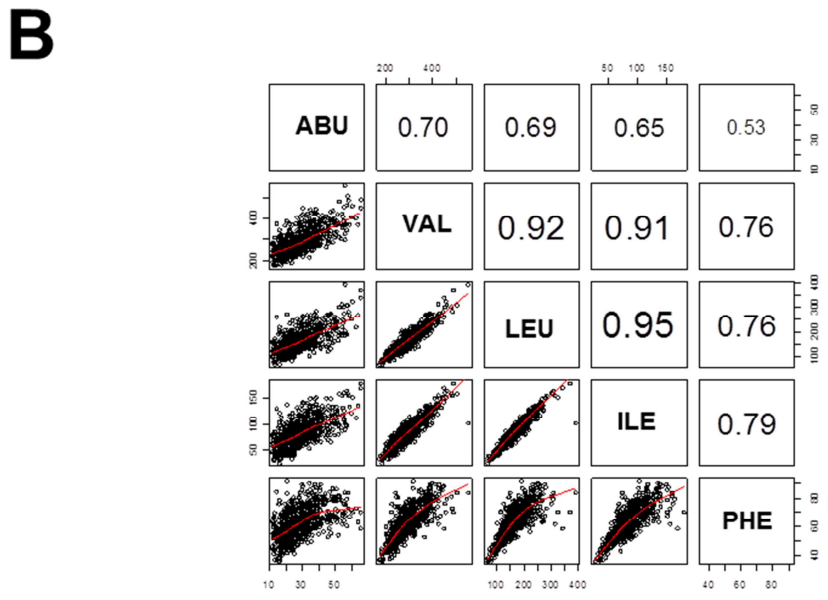


Figure 8: Concentration profiles and correlation matrix of selected plasma amino acids

(A) Concentration profiles of selected amino acids throughout the 4 day time course with embedded challenges. Data presented as mean ± SD. (B) Correlation matrix of a subset of plasma amino acids over all measured time-points of the entire 4 days of the HuMet-study. Strength of the association between two metabolites is represented by font size of the correlation coefficient r.

Abbreviations: ABU, α-aminobutyrate; CET, cycling ergometer test; ILE, isoleucine; LEU, leucine; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; PHE, phenylalanine; SLD, standard liquid diet; VAL, valine.

Plasma concentrations of 1-M-histidine decreased over the time-course irrespective of the metabolic challenge

Because of ethical reasons (see also 4.3.2; Figure 36), the HuMet-study had to be divided into two blocks. Block 1 consisted of the time-points 1-20 and block 2 consisted of the time-points 21-50. Both blocks spanned a period of 32 hours. Interestingly, independent of the challenge tests performed during the two study blocks, reductions of 1-M-histidine concentrations were observed (Figure 9).

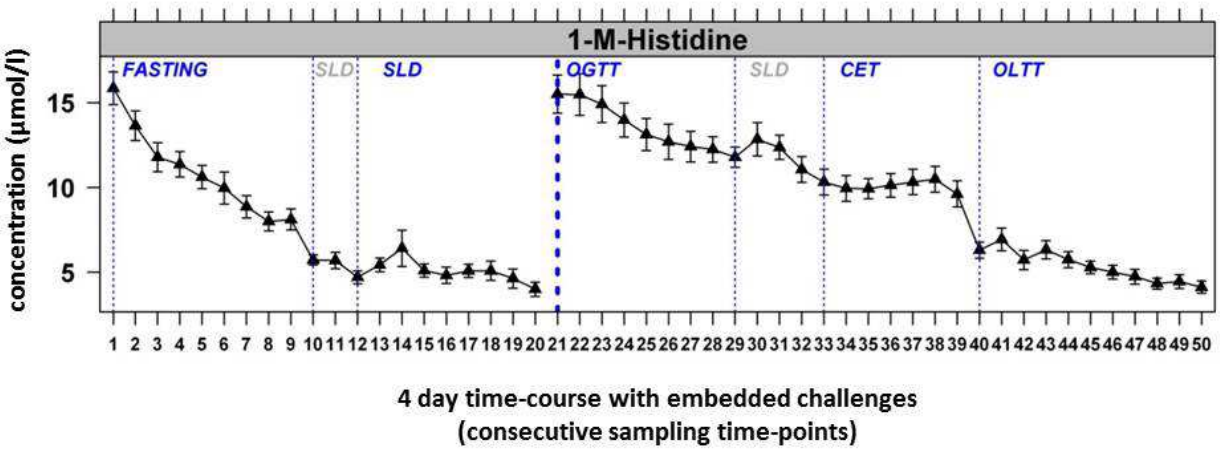


Figure 9: Plasma profile of 1-M-histidine over 4 days
Data are displayed as mean ± SEM. Vertical blue lines indicate new challenge; thicker dashed line separate block 1 of the study (time-points 1 - 20) from block 2 (time-points 21 – 50).
Abbreviations: CET, cycling ergometer test; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; SLD, standard liquid diet.

At the beginning and at the end of each challenge block, plasma concentrations were almost similar (day 1, time-point 1: 15.85 ± 3.71 µmol/l vs. day 3, time-point 21: 15.50 ± 4.34 µmol/l; day 2: time-point 20: 4.00 ± 1.60 µmol/l vs. day 4, time-point 50: 4.12 ± 1.39 µmol/l). Mean reduction of plasma concentrations over 32 hours was 11.85 ± 3.27 µmol/l for block 1 and 11.38 ± 3.29 µmol/l for block 2 (p = 0.697). The elimination rate over the 32 hours of each block was 0.370 ± 0.102 µmol/l per hour in block 1 and 0.355 ± 0.103 µmol/l per hour in block 2 (p = 0.697) and therefore constant.

Profile of 1-M-histidine in urine was similar to that in plasma

Similar to the time-dependent reduction in plasma levels, urinary 1-M-histidine concentrations (Figure 10) declined over both blocks of the HuMet-study as well. Surprisingly, there was no correlation between urinary and plasma 1-M-histidine concentrations, neither for the whole data set (r = 0.091, p = 0.173), nor when correlation analysis was performed using mean concentrations (r = 0.38, p = 0.162).

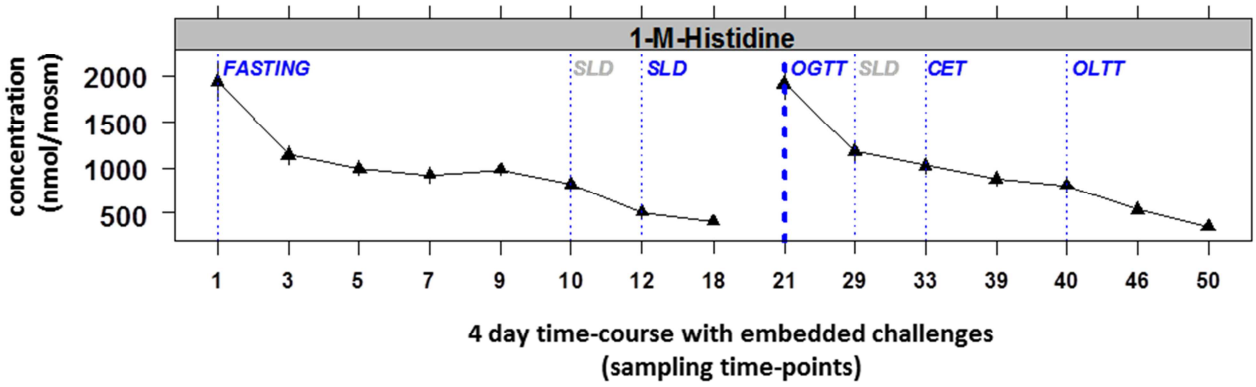


Figure 10: Urine profile of 1-M-histidine over 4 days
Data is displayed as mean ± SEM. Vertical blue lines indicate new challenge; thicker dashed line separate block 1 of the study (time-points 1 - 18) from block 2 (time-points 21 – 50).
Abbreviations: CET, cycling ergometer test; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; SLD, standard liquid diet.

Like for 1-M-histidine, correlation analysis of the urine and plasma amino acid data set did not reveal any association between metabolite concentrations of both compartments when the threshold was set to r = |0.5|. However, like in plasma, BCAA and α-aminobutyrate were highly correlated in urine (0.76 < r < 0.94), which was also revealed by hierarchical clustering analysis (HCA), as these metabolites were grouped (Figure 11). A second cluster was composed of the aromatic amino acids phenylalanine, tyrosine and tryptophan, a group of amino acids that was also highly correlated to each other (0.81 < r < 0.86).

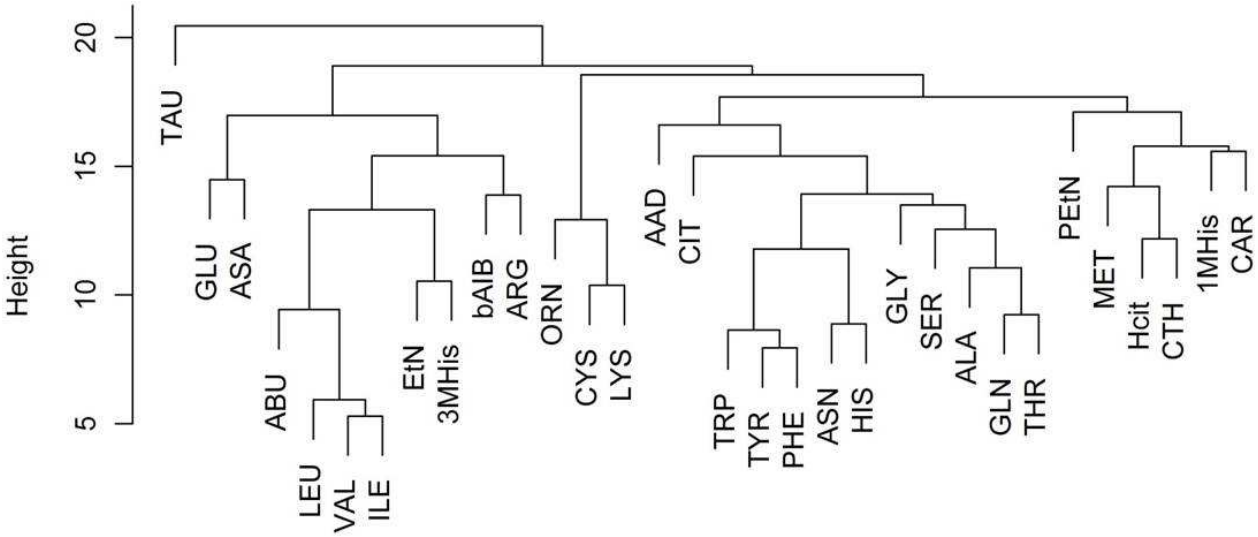


Figure 11: Hierarchical clustering analysis (euclidean distance, average linkage) of the urinary amino acid data set

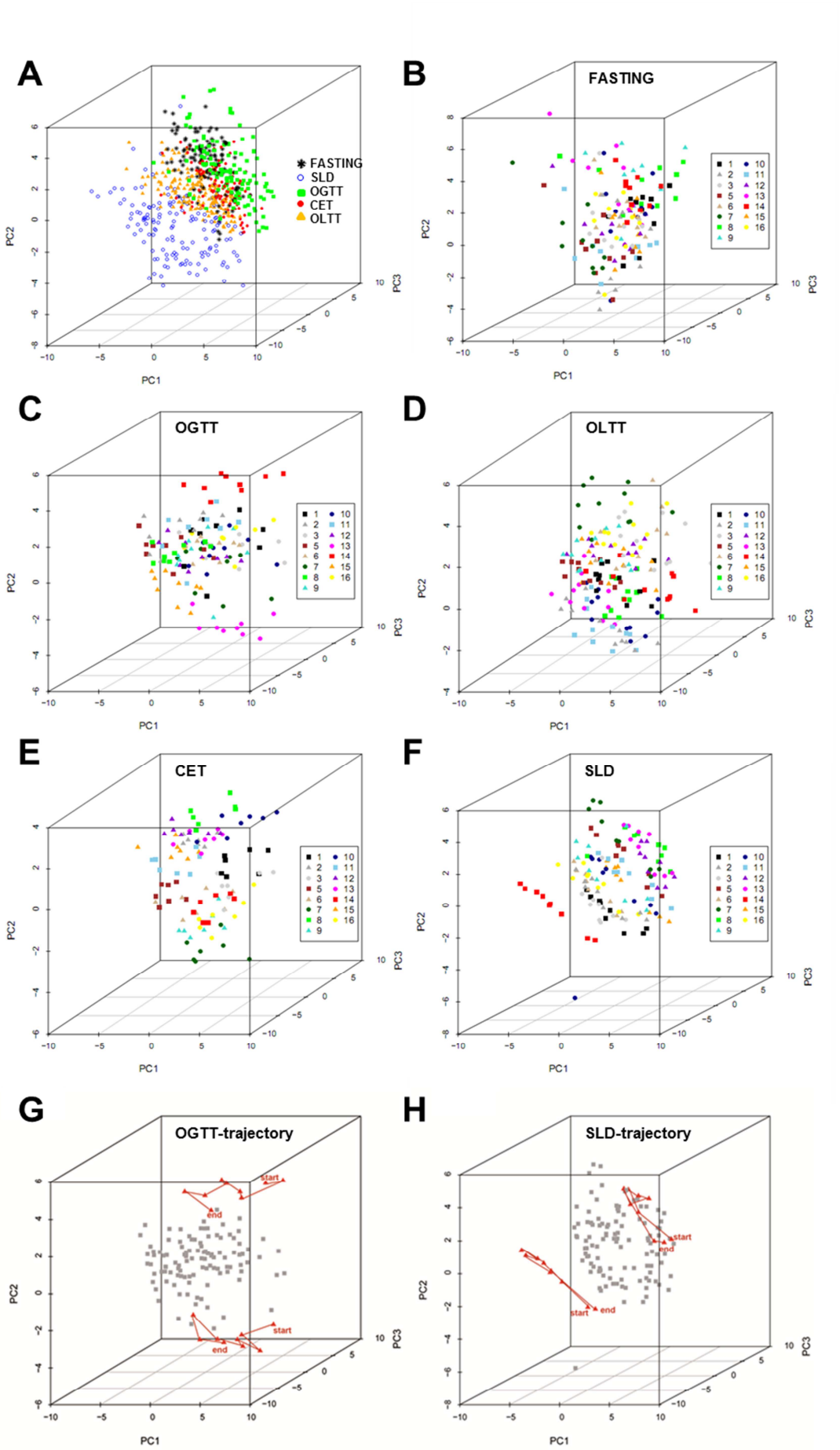
Abbreviations: 1MHis, 1-methyl-histidine; 3MHis, 3-methyl-histidine; AAD, α-aminoadipic acid; ABU, α-aminobutyrate; ALA, alanine; ASN, asparagine; ARG, arginine; ASA, argininosuccinate; bAIB, β-aminoisobutyrate; CAR, carnosine; CTH, cystathionine; CYS, cystine; CIT, citrulline; EtN, ethanolamine; GLN, glutamine; GLU, glutamate; GLY, Glycine; HCIT, homocitrulline; HIS, histidine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; ORN, ornithine; PEtN, P-ethanolamine; PHE, phenylalanine; PRO, proline; SAR, sarcosine; SER, serine; TAU, Taurine; THR, threonine; TRP, tryptophan; TYR, tyrosine; VAL, valine.

Challenges are reflected in the plasma amino acid metabolome

For the analysis of the plasma amino acid data set, we further applied multivariate statistical methods. Therefore, the whole data set was scaled to ensure equal distribution of variance. Principal component analysis (PCA) was performed over all measured time-points of the five metabolic challenges and revealed a challenge-dependent grouping (Figure 12A). However, in contrast to previously published data [98], which was mainly based on acylcarnitines, phosphatidylcholine species and sphingomyelins, the whole amino acid data set did not reveal an individual plasma amino acid profile (data not shown).

Figure 12: PCA score plots of metabolic challenges in the HuMet-Study

(A) Principle component analysis (PCA) was performed using the entire amino acid data set of the five metabolic challenges. Score plot reveals a grouping of the challenges by PCA. (B-F) Score plots of amino acid data subset. (B) There was no grouping observed for the 36 hours fasting challenge for samples of each subjects. Other metabolic challenges like (C) the oral glucose tolerance test (OGTT), (D) the oral lipid tolerance test (OLTT), (E) the cycling ergometer test (CET), and (F) the standard liquid diet (SLD) led to a subject specific grouping by the PCA. (G) Time-dependent trajectory of two volunteers through the metabolic space during the OGTT. At the end of the challenge (240 min after glucose administration), volunteers do not reach their original space. (H) Time-dependent trajectory of two volunteers through the metabolic space during the SLD. The metabolic space at the beginning and 240 min after the challenge are similar for each individual.


A
FASTING
SLD
OGTT
CET
OLTT
B
FASTING
C
OGTT
D
OLTT
E
CET
F
SLD
G
OGTT-trajectory
start
end
H
SLD-trajectory
PC1
PC2
PC3

Nevertheless, when challenge-specific data subsets were used, PCA revealed an individual amino acid profile for the OGTT, the OLTT, the CET and the SLD (Figure 12C-F), but not for the extended fasting challenge (Figure 12B).

As described by Krug *et al.* the metabolic challenges provoke a distinct time-dependent trajectory in the plasma acylcarnitine and phospholipid levels [98]. A clear trajectory for individuals' amino acid profiles were also found for instance for the anabolic challenges OGTT and SLD. For both challenges, a clear subject specific movement through the space is visible (Figure 12GH). It is of note, that for the SLD the individuals' profile returned to the original space 240 min post challenged. However, when the OGTT is considered, the metabolome failed to reach the original space 240 min after glucose ingestion.

Cycling ergometer test

To investigate the effects of physical activity on the amino acid metabolome, volunteers cycled on a stationary ergometer at their individual anaerobic threshold (75% maximal aerobic capacity (VO_2max)) for 30 min. Alanine concentrations increased during physical exercise and displayed peak concentrations at t = 30 min (33% increase, Figure 13A). When volunteers were cycling, glutamate concentrations remained unchanged, but increased 30 min post-exercise (73% increase). Although glutamine increased only slightly (10% increase), correlation analysis revealed an association between mean concentrations of alanine and glutamine (r = 0.81, p = 0.029). Alanine was further highly correlated with lactate (Figure 13B), that peaked at t = 30 min (494% increase, Figure 13A). Furthermore, high mean correlations were found between BCAA (valine vs. leucine, r = 0.93, p = 0.003; valine vs. isoleucine, r = 0.91, p = 0.005; leucine vs. isoleucine, r = 0.85, p = 0.016) and cationic amino acids such as ornithine, lysine, and arginine (ornithine vs. lysine, r = 0.97, p < 0.001; ornithine vs. arginine, r = 0.96, p < 0.001; arginine vs. lysine, r = 0.95, p = 0.001). When HCA was performed, these metabolites also grouped confirming the correlation analysis (Figure 13C).

The increased levels of alanine and glutamine may suggest an enhanced flux from skeletal muscle to liver for gluconeogenesis in the exercise state and indicated enhanced proteolysis. In order to estimate, if during the CET energy is also provided via lipolysis and hence β-oxidation, alanine, glutamate, glutamine and lactate concentrations were correlated with plasma acylcarnitine concentrations.

Acylcarnitines originate from β-oxidation in skeletal muscle (see scheme Figure 5). However, we found significant correlations only between alanine, glutamine, lactate and the acylcarnitine species C3, C4, C9 and C8:1 ranging from 0.8 < r < 0.9. In contrast, concentrations of acylcarnitines with a carbon-chain length > 10 were not associated with alanine, glutamine and lactate concentrations (see Suppl. Figure 39).

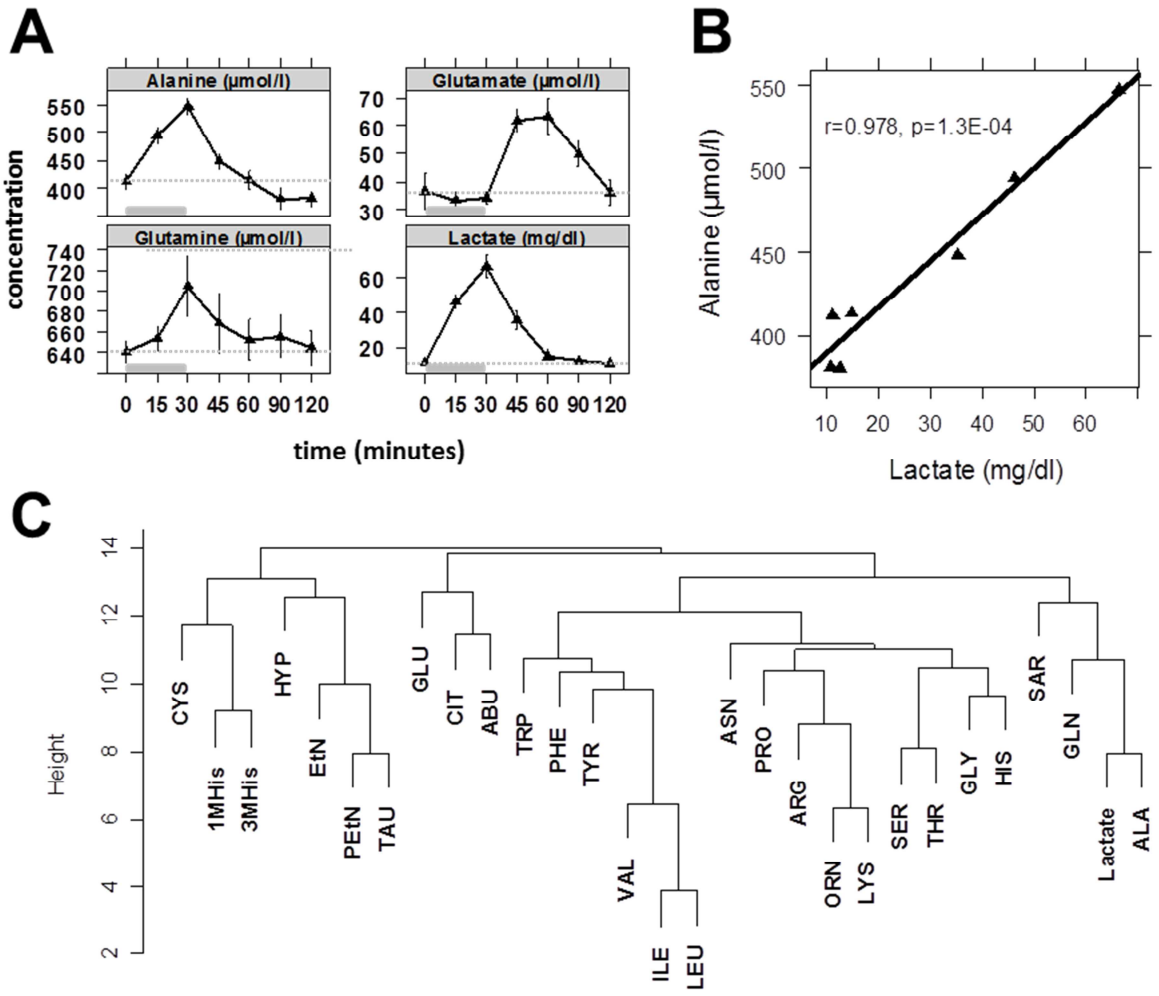


Figure 13: Cycling ergometer test (CET)
(A) Selected amino acids and lactate concentrations during the CET. Data are displayed as mean ± SEM. The grey bars illustrate the 30 min of exercise. (B) Correlation between mean plasma concentrations of alanine and lactate. Each triangle represents one timepoint (t=0, 15, 30, 45, 60, 90, 120 min). (C) Hierarchical clustering analysis (Euclidean distance, average linkage) of the amino acid data set including lactate concentrations for the CET.
Abbreviations: 1MHis, 1-methyl-histidine; 3MHis, 3-methyl-histidine; ABU, α-aminobutyrate; ALA, alanine; ASN, asparagine; ARG, arginine; CYS, cystine; CIT, citrulline; EtN, ethanolamine; GLN, glutamine; GLU, glutamate; GLY, glycine; HIS, histidine; HYP, hydroxyproline; ILE, isoleucine; LEU, leucine; LYS, lysine; ORN, ornithine; PEtN, P-ethanolamine; PHE, phenylalanine; PRO, proline; SAR, sarcosine; SER, serine; TAU, Taurine; THR, threonine; TRP, tryptophan; TYR, tyrosine; VAL, valine.

Anabolic and catabolic conditions induced by an oral glucose tolerance test and a 36 hours fasting period

The OGTT is highly standardized [74] and the most simplistic anabolic challenge. Single administration of glucose enables to investigate the anabolic response pattern without further implying confounding factors like different nutritional compounds or alterations in gastric emptying by dietary components. In response to glucose ingestion blood glucose concentrations as well as insulin levels peaked as expected after 30 min (glucose: 58% increase; insulin: 791% increase; Figure 14A).

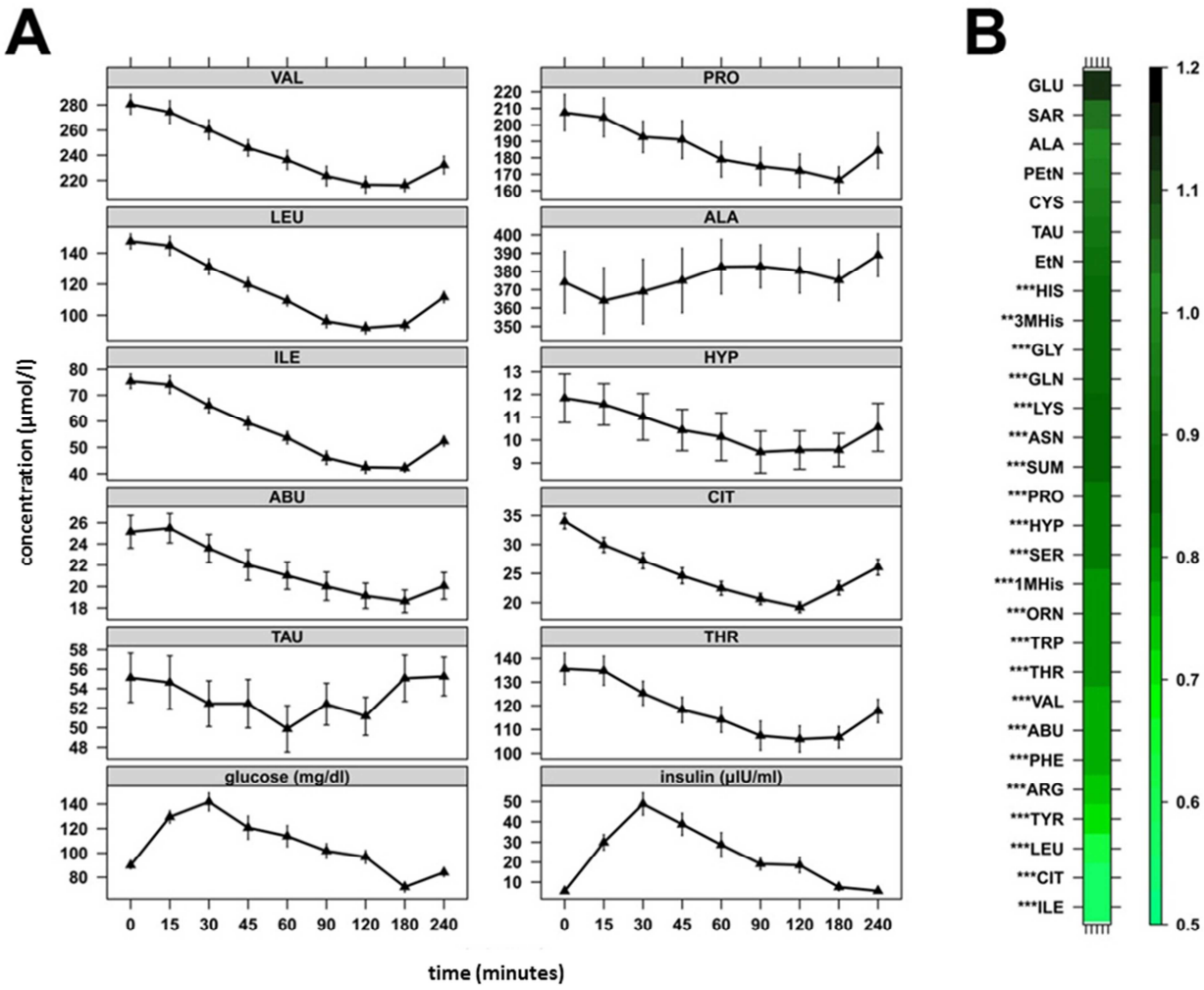


Figure 14: Plasma glucose, insulin and amino acid responses to an OGTT
(A) Time-courses of selected amino acids, blood glucose and insulin concentrations during the OGTT. (B) Levelplot of fold-change of t = 120 min compared to baseline (t = 0 min). Data are displayed as mean ± SEM. Asterisks indicate statistical significance based on repeated-measurement ANOVA following Tukey post-hoc test. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Abbreviations: see Figure 13.

However, the OGTT induced a reduction in plasma concentrations for 75% of the amino acids (Figure 14AB). Isoleucine (fold-change (FC) = 0.56 ± 0.049), leucine (FC = 0.62 ± 0.053), but also the urea cycle intermediate citrulline (FC = 0.56 ± 0.052) were upon the most responsive amino acids after glucose ingestion (Figure 14B). Interestingly, even 240 min after glucose administration, most amino acids concentrations remained reduced compared to t = 0 min and for 40% of the amino acids the FC was still lower than 0.85.

In contrast to the OGTT, an extended fasting period over 36 hours induced a divergent response of the amino acid metabolome, as about 28% and 32% of the amino acids increased or decreased, respectively (Figure 15AB).

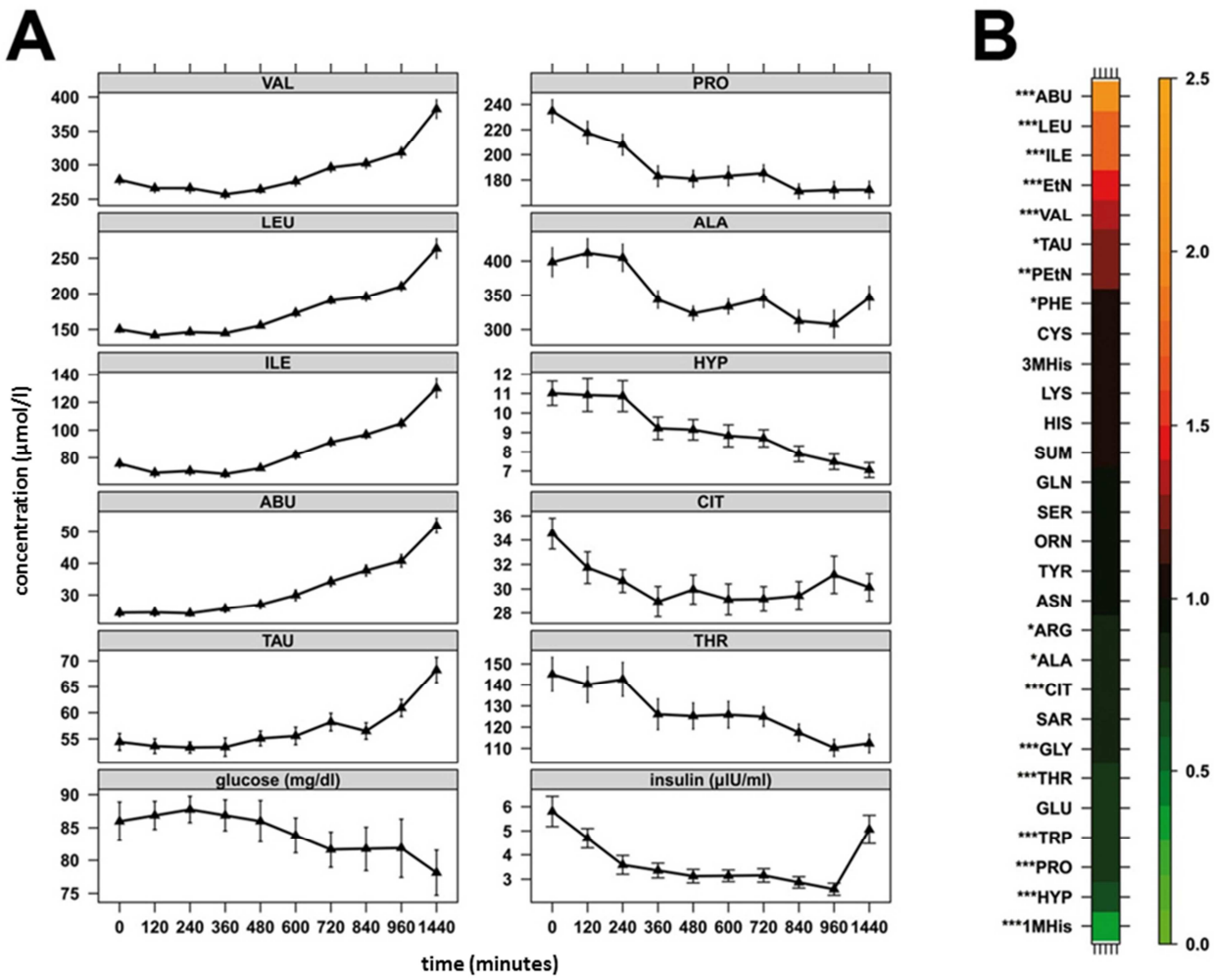


Figure 15: Plasma glucose, insulin and amino acid responses to fasting

(A) Time-courses of selected amino acids, blood glucose and insulin concentrations during extended fasting. (B) Levelplot of fold-change of t = 1440 min compared to baseline (t = 0 min). Data are displayed as mean ± SEM. Asterisks indicate statistical significance based on repeated-measurement ANOVA following Tukey post-hoc test. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Abbreviations: see Figure 13.

The two derivatives α-aminobutyrate (FC = 2.19 ± 0.418) and 1-M-histidine (FC = 0.36 ± 0.08) depicted the magnitude of metabolic responses (Figure 15B).
As shown in Figure 10 1-M-histidine declined independent of the challenges applied, suggesting that this reduction of plasma concentrations was not induced by the fasting challenge itself. Repeated-measurement ANOVA revealed that only cystine, glutamate and sarcosine were non-responsive to both metabolic states, whereas eight metabolites (tryptophan, arginine, citrulline, glycine, hydroxyproline, proline, threonine and 1-M-histidine) displayed congenerous decreases during fasting and the OGTT.

The BCAA, α-aminobutyrate and phenylalanine showed opposed responses to fasting and the OGTT (Figure 14B, Figure 15B) and thus might be potential markers for the specific metabolic condition. As already shown in Figure 8B, these metabolites and in particular α-aminobutyrate and the BCAA species were highly associated in the entire 4-day study period. Considering the individuals' responses to fasting and the OGTT the correlation between α-aminobutyrate and the sum of BCAA is even higher (Table 3) and could be confirmed by the average response as well (Fasting: r=0.987, *p*=1.20E-07 and OGTT: r=0.987, *p*=8.18E-07).

Table 3: Pearson correlations between α-aminobutyrate and sum of BCAA during fasting and OGTT

	Fasting		OGTT	
Individual	**r**	***p*-value**	**r**	***p*-value**
1	0.841	0.002	0.981	3.30E-06
2	0.930	9.41E-05	0.978	4.98E-06
3	0.937	6.47E-05	0.890	0.001
5	0.969	3.74E-06	0.947	1.1E-04
6	0.966	5.74E-06	0.851	0.004
7	0.969	3.99E-06	0.811	0.008
8	0.930	9.82E-05	0.967	2.00E-05
9	0.957	1.39E-05	0.918	4.80E-04
10	0.959	1.14E-05	0.853	0.003
11	0.992	1.91E-08	0.935	2.2E-04
12	0.923	1.37E-04	0.833	0.005
13	0.95	2.63E-05	0.928	3.09E-04
14	0.898	4.16E-04	0.919	4.65E-04
15	0.956	1.51E-05	0.806	0.009
16	0.941	4.82E-05	0.924	3.73E-04
Average	0.987	1.20E-07	0.987	8.18E-07

Previously, α-aminobutyrate was suggested as a potential fasting marker [82] and the increase of BCAA in the catabolic state was already described in 1969 by Felig *et al.* [100]. As mentioned in the introduction section, BCAA are considered as potential biomarker for the diabetic state. Recently, Newgard hypothesized that the catabolism of BCAA is affected in the state of obesity and type 2 diabetes resulting in the observed elevations [55]. Propionyl-carnitine (C3) and valeryl-carnitine (C5) are acylcarnitine species that can be derived from BCAA catabolism and were described as discriminating metabolites for the diabetic state as well [55]. Thus, we addressed by correlation analysis, if BCAA can be linked to their catabolites C3 and C5 and/or to other acylcarnitine species in the fasting state. The time-dependent changes in the fasting challenge were similar between free carnitine (C0) and C3 (Figure 16A).

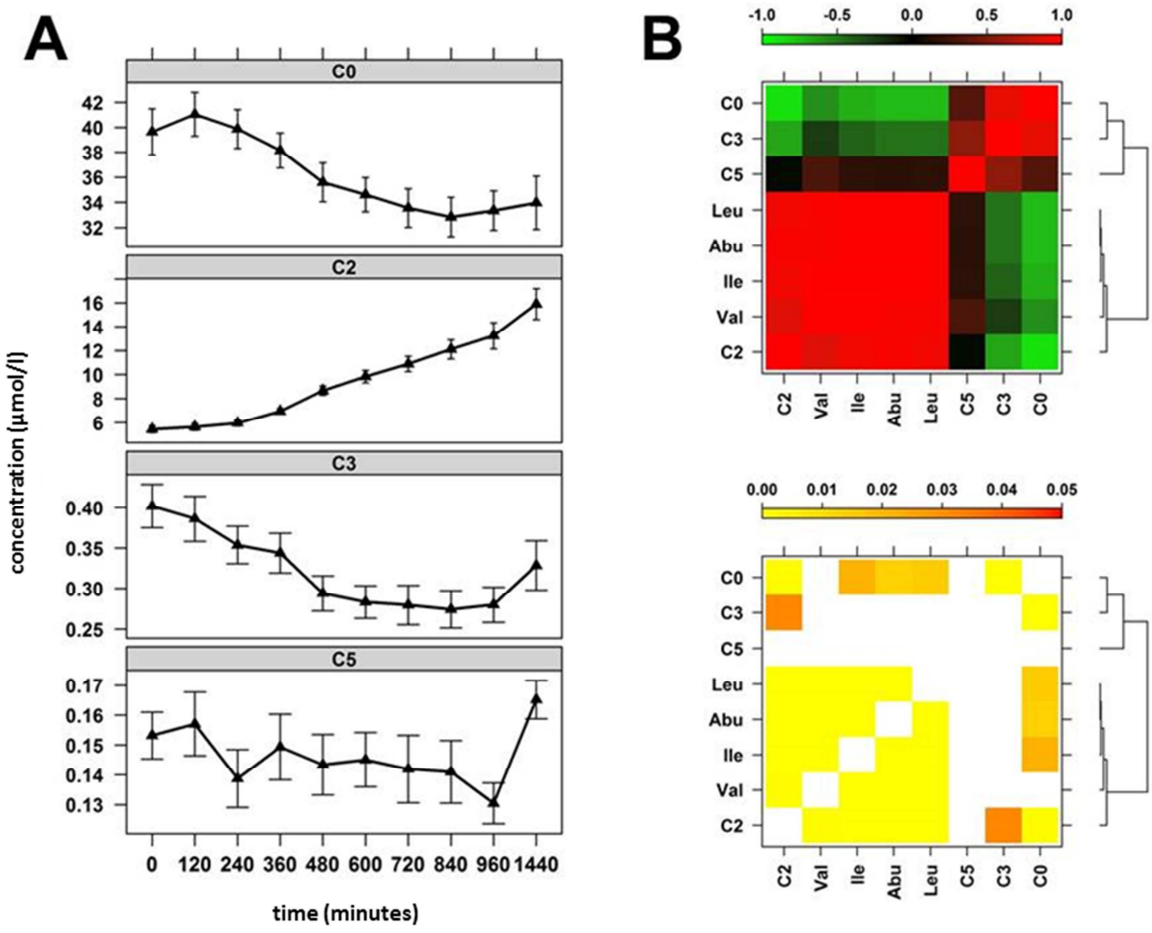


Figure 16: Selected acylcarnitine species during fasting challenge and their mean correlations with BCAA and α-aminobutyrate

(A) Time-course profiles of selected acylcarnitine species. Data are displayed as mean ± SEM. (B) Pearson correlation matrix between mean concentrations of selected acylcarnitine species, BCAA and α-aminobutyrate (top) and the corresponding matrix displaying the *p*-values of the correlation matrix (bottom).

However, only C0 was highly negatively associated to leucine, isoleucine and α-aminobutyrate, but not C3 (Figure 16B). C5 did show neither a clear response to fasting nor any association to BCAA and α-aminobutyrate. The plasma response of acetyl-carnitine (C2) is similar to that of BCAA and α-aminobutyrate and this distinct time-dependent pattern is reflected in the high correlations between these metabolites (Figure 16B). In addition, acylcarnitine species with a carbon-chain length between 10 and 18 were highly associated with BCAA and α-aminobutyrate levels (data not shown).

After glucose administration, concentrations of C2 were reduced and lowest levels were measured 120 min post challenge (Figure 17A).

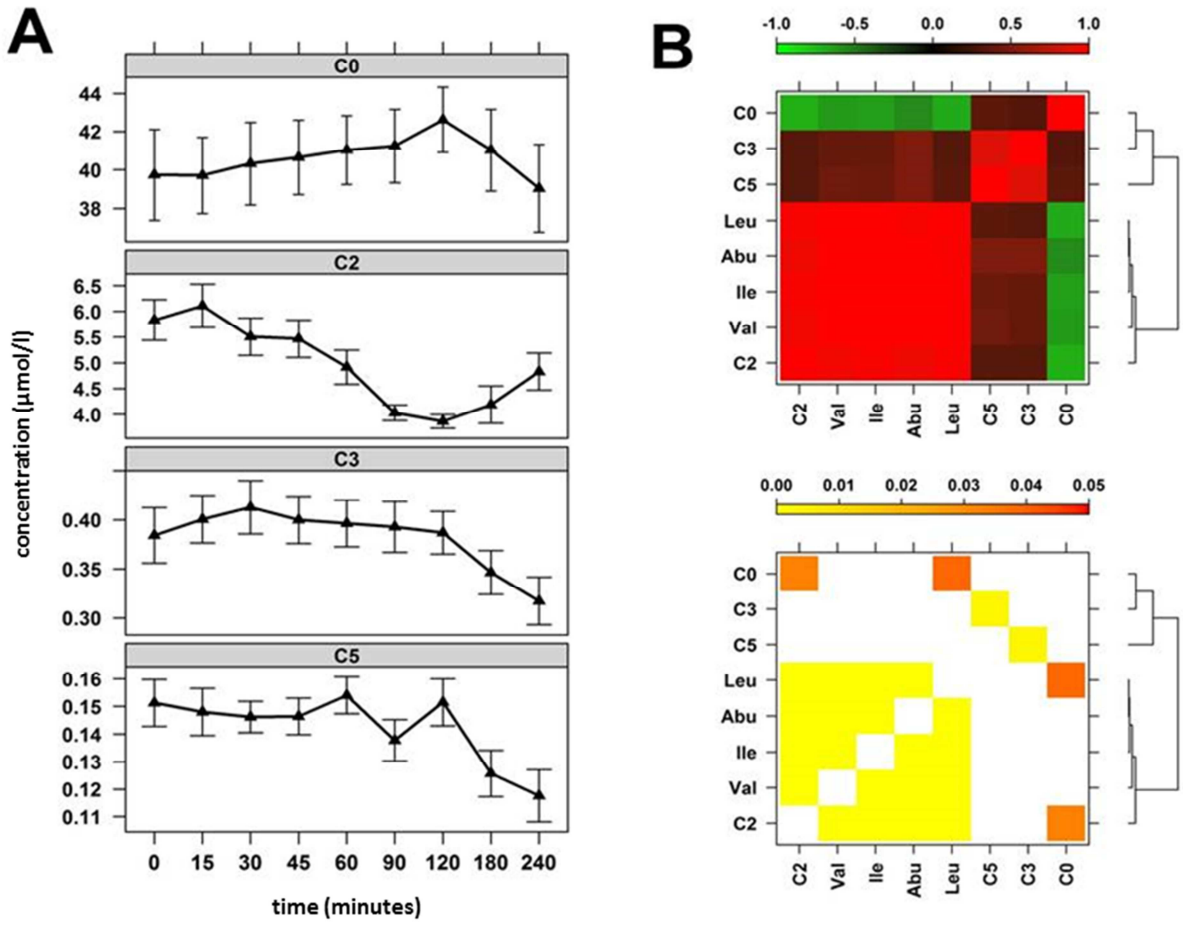


Figure 17: Selected acylcarnitine species during OGTT and their mean correlation to BCAA and α-aminobutyrate

(A) Time-course profiles of selected acylcarnitine species. Data are displayed as mean ± SEM. (B) Pearson correlation matrix between mean concentrations of selected acylcarnitine species, BCAA and α-aminobutyrate (top) and the corresponding matrix displaying the *p*-values of the correlation matrix (bottom).

Thus, the time-profile was highly associated with changes that were observed for the majority of the plasma amino acids, as shown by high positive correlations between C2, the BCAA and α-aminobutyrate (Figure 17B). No correlations were found between BCAA, α-aminobutyrate, C3 and C5, while C0 was again negatively associated with the investigated amino acids. However, only the correlation between C0 and plasma leucine displayed a significant *p*-value < 0.05 (Figure 17B).

C2 originates from β-oxidation as it is formed from acetyl-CoA. As we could show that mean correlations of C2 were highly associated to amino acids that are proposed as fasting markers, we further asked, whether this correlation can also be observed on an individual level. We therefore used the FC of t = 1440 min (36 hours fasting) over baseline (t = 0 min) concentrations for correlation analysis. These values reflected the maximal response to the catabolic state of each individual. As shown in Figure 18, we found high associations between FC of C2 and FC of valine, leucine, isoleucine as well as α–aminobutyrate. Depicted as red indices, the same subgroup of individuals displayed the lowest respectively highest metabolic response to the catabolic condition for the metabolites shown. This suggested, that the adaptation to the fasting state underlay a metabotype specific response patterns.

The whole data set of the HuMet-study is depicted in the Appendix A, Suppl. Tables 14-22 according to challenges: For amino acids following tables (Number of table): Fasting plasma (14), OGTT plasma (15), SLD plasma (16), CET plasma (17), OLTT plasma (18), urine (19). For acylcarnitines the following tables: CET (20), Fasting (21), OGTT (22).

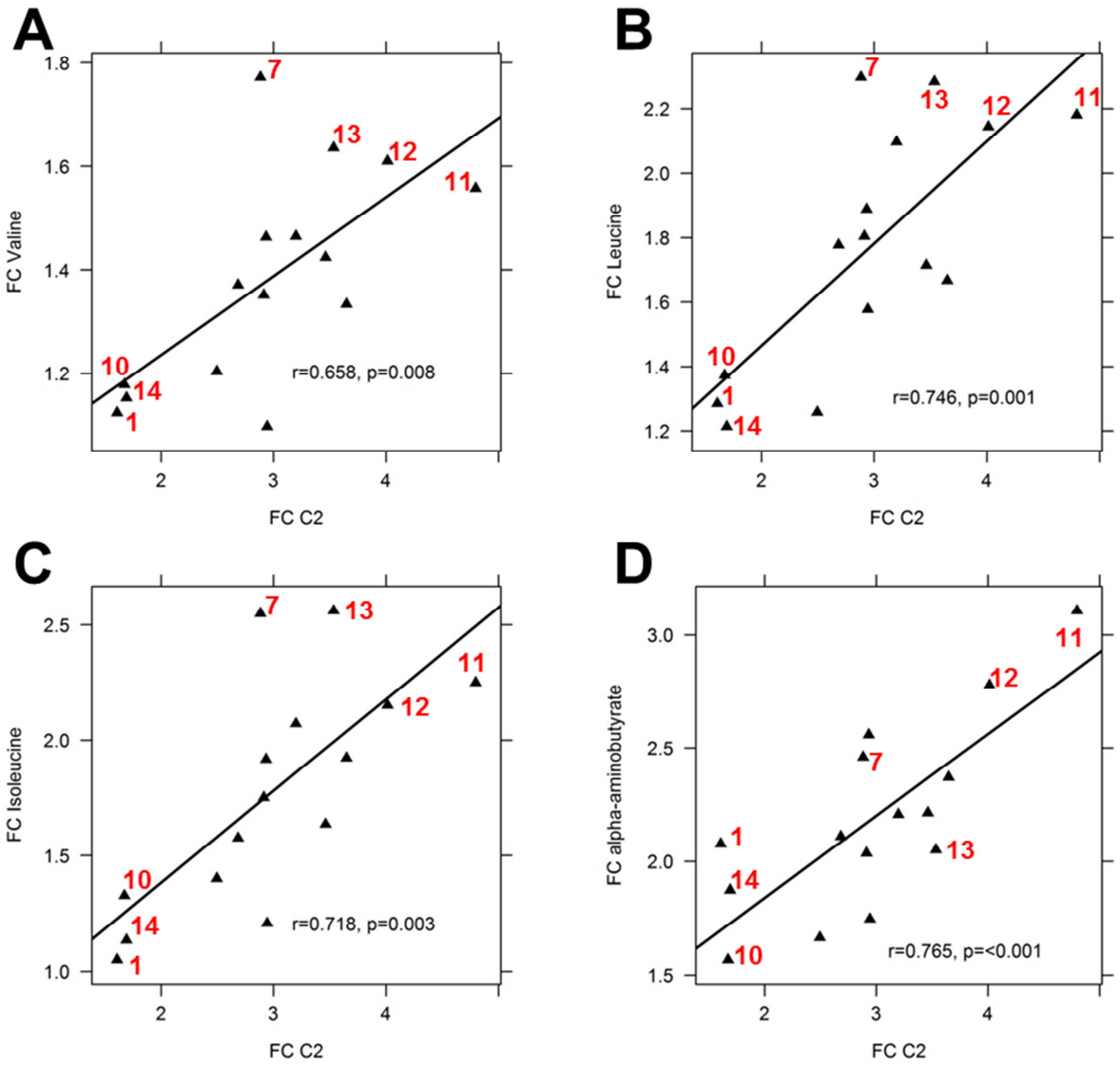


Figure 18: Pearson correlations between FC of C2 and FC of valine (A), leucine (B), isoleucine (C) and α-aminobutyrate (D)

Fold-change (FC) of plasma acetylcarnitine (C2), valine, leucine, isoleucine and α-aminobutyrate concentrations of t=1440 min over baseline values (t=0 min). Red numbers indicate selected individuals.

2.1.2. Mouse studies

In the majority of human trials metabolite profiling is limited to plasma, urine or in view cases also saliva. Whereas these bio fluids can be sampled non-invasively, samples such as biopsies are more difficult to obtain. In addition, each bio fluid represents just one compartment of the whole organism. As we could show in the HuMet-study, especially plasma reflects a highly dynamic continuum that responses quickly to changes of the metabolic state. We therefore tried to overcome these limitations by using mouse models that would allow investigating the metabolic response pattern in the anabolic and catabolic state on tissue levels. By assessing the amino acid response to an OGTT and fasting in mice, we consequently also profiled tissues such as liver or skeletal muscle.

The metabolite responses to an OGTT in mice

Mice were gavaged with a 20% glucose solution that simulated the OGTT in humans. Amino acid levels in plasma, skeletal muscle and liver were determined at baseline (0 min) and after 30, 60 and 120 minutes. Blood glucose concentrations increased 30 min after glucose load (Figure 19, p = 0.002). When we applied multivariate statistics like PCA and HCA on the amino acid data sets of plasma, skeletal muscle and liver, we could not detect any clustering based on the different time-points (data not shown).

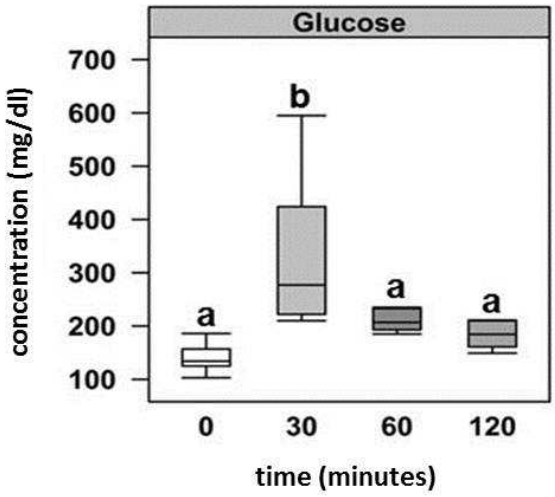


Figure 19: Blood glucose concentration after an OGTT in mice
P-values obtained by one-way ANOVA. N=6 per group. Different subscript letters indicate statistical significance (p < 0.05) based on Tukey post-hoc analysis to compare between groups.

Plasma. Between-group comparisons based on one-way ANOVA analysis revealed that only 21% of all measured plasma amino acids displayed significant changes induced by the oral glucose load (Figure 20 and Figure 21B).

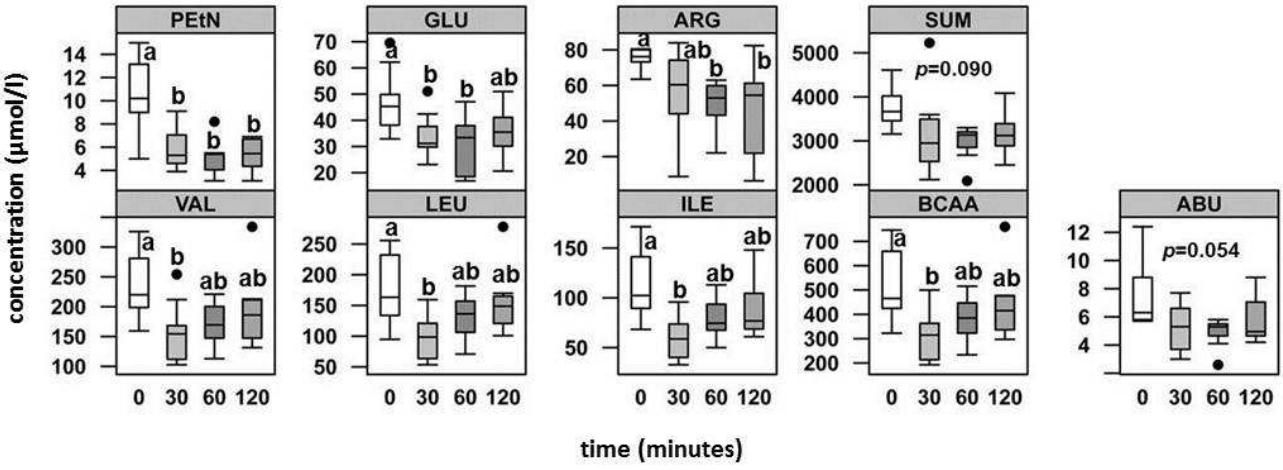


Figure 20: Significantly altered plasma amino acid levels in mice after an oral glucose load measured by LC-MS/MS

Mice were gavaged with 300 µl of 20% glucose solution or with water (0 min). Plasma amino acids were determined after 0 min, 30 min, 60 min and 120 min, respectively. N=7-11 per group. Dots represent outliers. *P*-values obtained by one-way ANOVA adjusted with Benjamini-Hochberg correction. Different subscript letters indicate statistical significance ($p < 0.05$) based on Tukey post-hoc analysis to compare between groups.
Abbreviations: ABU, α-aminobutyrate; ARG, arginine; BCAA, branched-chain amino acids; GLU, glutamate; ILE, isoleucine; LEU, leucine; SUM, total sum of amino acids; PEtN, P-ethanolamine; VAL, valine.

Similar to the human trial, valine, leucine and isoleucine were amongst the most responsive amino acids and declined after glucose load. Furthermore, α-aminobutyrate concentrations (p = 0.054) as well as glutamate, arginine and P-ethanolamine concentrations were reduced. As depicted in the levelplot (Figure 21A) and indicated in the total sum of all amino acids (p = 0.090, see Figure 20), the whole plasma amino acid metabolome showed a tendency to a reduction of concentrations 30 minutes after glucose ingestion. Like for the human trial, we further investigated the response of an oral glucose administration on plasma acylcarnitine levels (see Suppl. Table 24). After 30 minutes, 37.5 % of the measured acylcarnitine species displayed a FC < 0.8, but this was not statistically significant based on one-way ANOVA. Nevertheless, C0 raised in concentrations after glucose administration (FC = 1.35, p = 0.097), while C2 displayed reduced concentrations (FC = 0.59, p = 0.184). Therefore, this response pattern is similar to the one found in the OGTT challenge of the HuMet-study.

Skeletal muscle and Liver. Oral glucose administration did not result in significant changes on tissue levels for the majority of amino acids (Figure 21BC). Only methionine determined in skeletal muscle showed an increased concentration 60 min after glucose ingestion (0 min: 1.38 ± 0.66 µmol/g protein, 30 min: 1.95 ± 0.48 µmol/g protein; 60 min: 2.84 ± 0.64 µmol/g protein; 120 min: 1.47 ± 0.19 µmol/g protein; p = 0.013). Like for plasma, the amino acid metabolome in liver and skeletal muscle displayed reduced concentrations 30 minutes post glucose loading. Yet, the overall response to the OGTT challenge is not as clear as it was observed for the plasma response in the human trial.

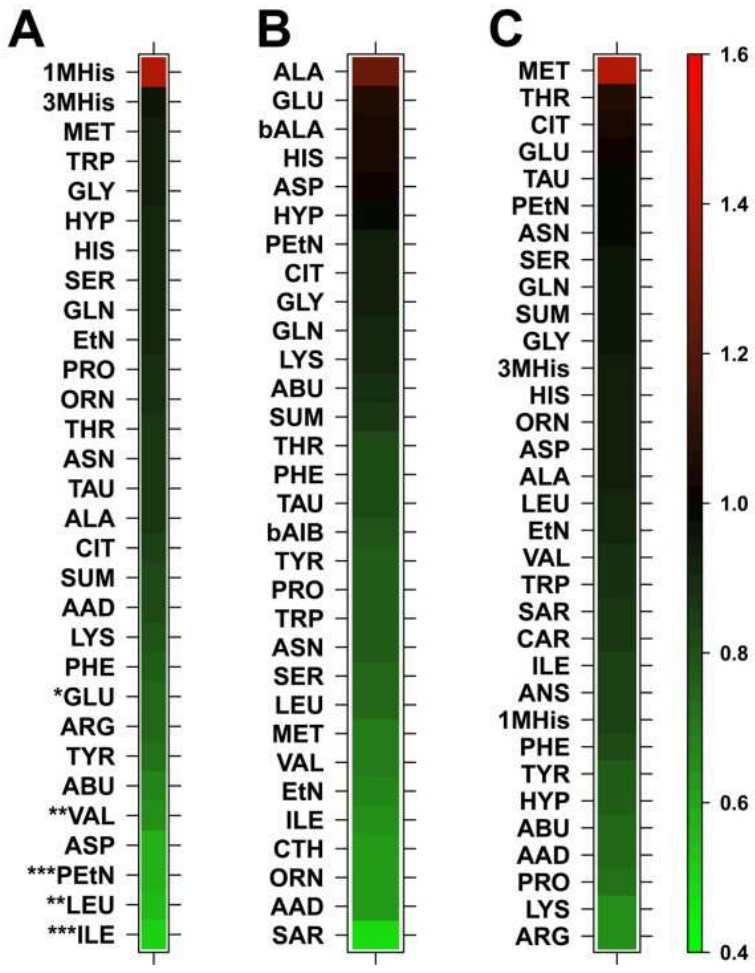


Figure 21: Levelplots showing the fold-change of plasma, liver and skeletal muscle amino acid levels 30 minutes after glucose administration in mice

P-values obtained by one-way ANOVA adjusted with Benjamini-Hochberg corrections. N=6-11 per group. Asterisks indicate statistical significance. *p < 0.05, **p < 0.01, ***p < 0.001 based on Tukey post-hoc analysis to compare between groups.

Abbreviations: 1MHis, 1-methyl-histidine; 3MHis, 3-methyl-histidine; AAD, α-aminoadipate; ABU, α-aminobutyrate; ALA, alanine; ANS, Anserine; ASN, asparagine; ASP, aspartate; ARG, arginine; bAla, β-alanine; bAIB, β-aminoisobutyrate; CAR, carnosine; CTH, cystathionine; CIT, citrulline; EtN, ethanolamine; GLN, glutamine; GLU, glutamate; GLY, glycine; HIS, histidine; HYP, Hydroxyproline; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; ORN, ornithine; PEtN, P-ethanolamine; PHE, phenylalanine; PRO, proline; SAR, sarcosine; SER, serine; TAU, taurine; THR, threonine; TRP, tryptophan; TYR, tyrosine; VAL, valine, SUM, total sum amino acids.

Plasma amino acids and acylcarnitines of the OGTT in mice are summarized in Suppl. Table 23 respectively Suppl. Table 24. Skeletal muscle and liver amino acids of the OGTT in mice are depicted in supplementary table Suppl. Table 25 respectively Suppl. Table 26.

The response to fasting in mice

In the fasting state, the interplay of different organs is essential to meet the energy requirements of the organism and we therefore investigated fasting-induced changes in plasma, skeletal muscle as well as liver in mice. A short-term fasting of 6 hours (control group) induced a weight loss of about 5% of the initial body weight. Mice that were fasted for 12 hours (F12) and 24 hours (F24) lost about 9% and 12% of their body weight (Table 4). Liver weight and blood glucose concentrations were reduced significantly in the F12 and F24 group compared to control mice. Noteworthy, animals of the F24 group did not lose additional liver weight and there was no further reduction of blood glucose concentrations compared to the F12 group (Table 4).

Table 4: Body weight, liver weight and blood glucose concentrations in mice after fasting

Mice (n=8 per group) were fasted either for 6 hours (control group), 12 hours over night (F12) or for 24 hours (F24).

	Control	F12	F24	*p*-value
% of initial body weight	95.27 ± 1.14	91.48 ± 1.03[a]	88.32 ± 2.27[ab]	<0.001
Liver weight (g)	1.16 ± 0.05	1.00 ± 0.08[a]	1.00 ± 0.07[a]	<0.001
Glucose fasting (mg/dl)	170.12 ± 28.81	79.75 ± 4.46[a]	81.62 ± 8.77[a]	<0.001

Data are presented as mean ± SD. p-value obtained by one-way ANOVA and adjusted using the Benjamini-Hochberg correction. [a]$p < 0.05$ obtained by Tukey post-hoc analysis compared to C group. [b]$p < 0.05$ obtained by Tukey post-hoc analysis compared to F12 group.

In contrast to the OGTT performed in mice, both, HCA and PCA as multivariate statistical approaches revealed a distinct grouping between control mice and fasted mice. Interestingly, there was no further clustering between both fasting groups (F12 and F24), neither for the whole data set (Figure 22A), nor when plasma, liver and skeletal muscle amino acid data were analyzed separately (Figure 22B-D).

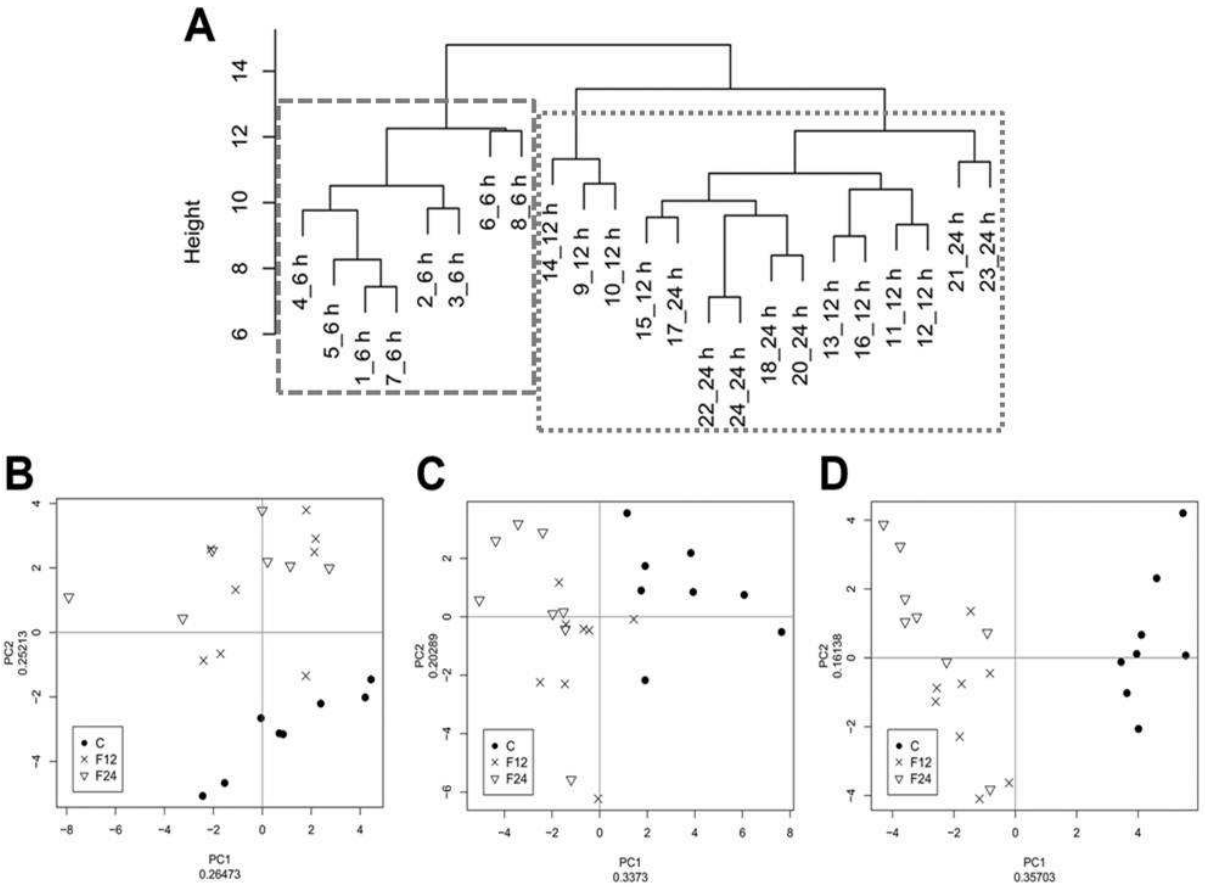


Figure 22: Hierarchical clustering analysis and PCA score plots from amino acid data of fasting mice

(A) Hierarchical clustering analysis tree (Euclidean distance, average linkage) including plasma, skeletal muscle and liver amino acid data. Coding: Mouse_Hours of fasting (6 h, 12 h, 24 h according to groups). Mouse 19 was excluded as plasma data is missing. (B-D) PCA score plot from amino acid data derived from plasma (B), liver (C), and skeletal muscle (D). Control mice (C, 6 hours fasted mice) were separated from the extended fasted groups F12 and F24 (mice fasted for 12 hours and 24 hours, respectively).

Plasma. In plasma, 53% of all measured metabolites displayed significant changes induced by fasting based on one-way ANOVA analysis (Figure 23). Like in the human trial, fasting led to higher plasma levels of valine, leucine, isoleucine and α-aminobutyrate. The aromatic amino acid phenylalanine was elevated in both fasting groups as well. Furthermore, threonine and 3-M-histidine increased, while alanine, hydroxyproline and arginine decreased during fasting. Taurine and α-aminoadipate concentrations were higher in the F12 group compared to controls. ^{1}H-NMR analysis revealed that concentrations of 2-OH-butyrate, creatine and ketoneogenesis-derived compounds like 3-OH-butyrate, acetate and acetoacetate were higher in fasted animals compared to controls. In contrast, the gluconeogenic substrates lactate and pyruvate were reduced in plasma of fasted animals compared to control animals (Figure 24).

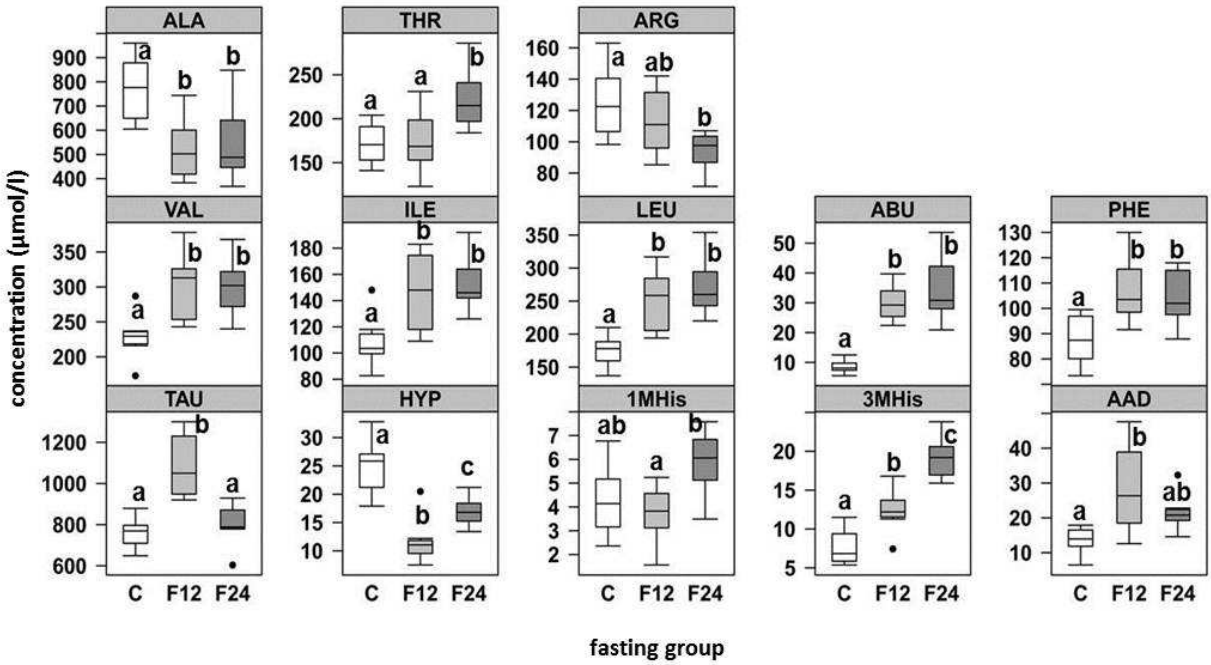


Figure 23: Significantly regulated plasma amino acids in fasted mice, measured by LC-MS/MS

Mice were either fasted for 6 hours (C), 12 hours over night (F12) or 24 hours (F24). N=7-8 per group. Dots represent outliers. *P*-values obtained by one-way ANOVA adjusted with Benjamini-Hochberg correction. Different subscript letters indicate statistical significance ($p < 0.05$) based on Tukey post-hoc analysis to compare between groups.

Abbreviations: 1MHis, 1-methyl-histidine; 3MHis, 3-methyl-histidine; AAD, α-aminoadipate; ABU, α-aminobutyrate; ALA, alanine; ARG, arginine; HYP, hydroxyproline; ILE, isoleucine; LEU, leucine; PHE, phenylalanine; TAU, taurine; THR, threonine; VAL, valine.

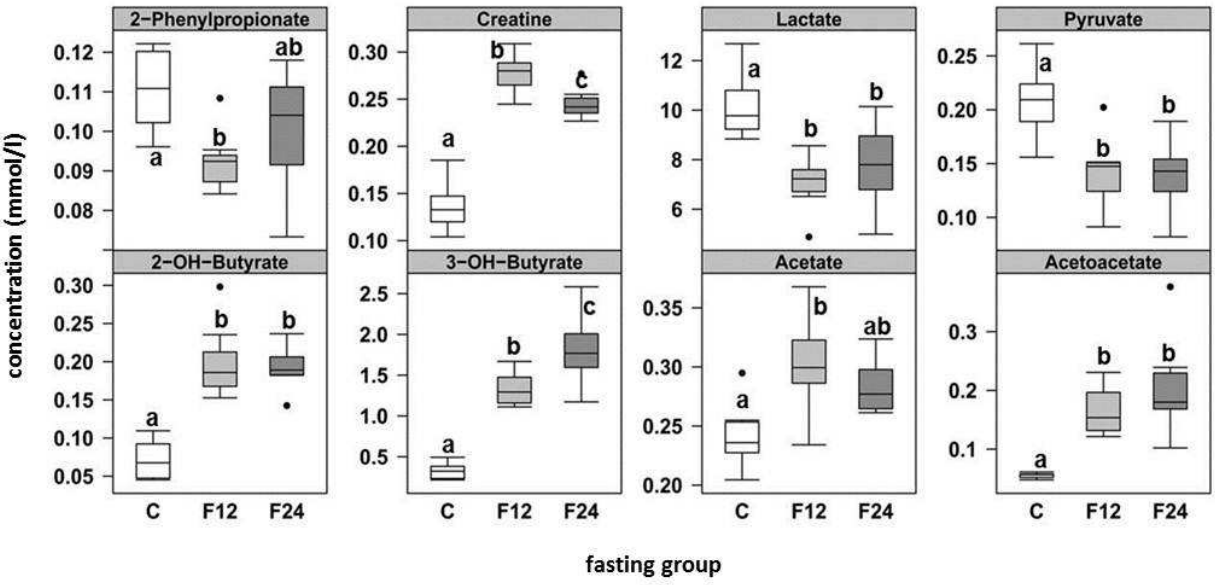


Figure 24: Significantly regulated plasma metabolites in fasted mice, measured by ^{1}H NMR

Mice were either fasted for 6 hours (C), 12 hours over night (F12) or 24 hours (F24). N=7-8 per group. Dots represent outliers. *P*-values obtained by one-way ANOVA adjusted with Benjamini-Hochberg correction. Different subscript letters indicate statistical significance ($p < 0.05$) based on Tukey post-hoc analysis to compare between groups.

We further determined acylcarnitine concentrations in plasma (Table 5). Like for the amino acid data sets, both fasting groups could not be distinguished by HCA and PCA. Nevertheless, there was a distinct separation of the fasting groups from the control group (data not shown). Core findings of the human fasting trial could be confirmed in the mouse experiment: Fasting induced a reduction of C0 levels (FC = 0.51 after 12 hours and FC = 0.47 after 24 hours) and an increase of C2 levels (FC = 1.33 after 12 hours and FC = 1.27 after 24 hours). The acylcarnitines C16:2, C18:1 and C18:2 displayed the most pronounced increases in concentrations (Table 5).

Table 5: Plasma acylcarnitine concentrations during fasting in mice
Mice were either fasted for 6 hours (C), 12 hours over night (F12) or 24 hours (F24). N=7-8 per group. Data is ordered based on FC of F24.

	C	F12		F24		
Acylcarnitine	**µmol/l**	**µmol/l**	**FC**	**µmol/l**	**FC**	***p*-value**
C0	19.36 ± 2.43	9.79 ± 1.13	0.51[a]	9.10 ± 1.78	0.47[a]	**<0.001**
C4:1	0.27 ± 0.04	0.20 ± 0.03	0.75	0.17 ± 0.09	0.62[a]	**0.010**
C3	0.58 ± 0.23	0.51 ± 0.24	0.87	0.45 ± 0.20	0.77	0.564
C10:2	0.05 ± 0.01	0.04 ± 0.00	0.95	0.04 ± 0.01	0.91	0.575
C5:1	0.13 ± 0.01	0.14 ± 0.01	1.06	0.13 ± 0.02	0.96	0.242
C5-OH (C3-M-DC)	0.16 ± 0.02	0.17 ± 0.01	1.07	0.15 ± 0.01	0.98	0.278
C3:1	0.03 ± 0.01	0.04 ± 0.00	1.07	0.03 ± 0.00	0.98	0.454
C8	0.17 ± 0.01	0.18 ± 0.02	1.08	0.17 ± 0.02	0.99	0.239
C3-OH	0.09 ± 0.01	0.09 ± 0.01	1.03	0.09 ± 0.01	0.99	0.833
C6:1	0.09 ± 0.01	0.10 ± 0.01	1.10	0.09 ± 0.02	1.00	0.225
C9	0.12 ± 0.01	0.13 ± 0.01	1.08	0.12 ± 0.02	1.00	0.278
C7-DC	0.07 ± 0.01	0.08 ± 0.01	1.12	0.07 ± 0.01	1.00	0.191
C12-DC	0.34 ± 0.03	0.36 ± 0.02	1.07	0.36 ± 0.05	1.04	0.487
C4	1.29 ± 0.73	1.20 ± 0.58	0.93	1.38 ± 0.64	1.06	0.871
C5	0.34 ± 0.06	0.42 ± 0.06	1.23	0.38 ± 0.08	1.10	0.109
C10:1	0.11 ± 0.01	0.13 ± 0.01	1.14[a]	0.12 ± 0.02	1.10	**0.031**
C5:1-DC	0.20 ± 0.02	0.22 ± 0.04	1.09	0.23 ± 0.02	1.12	0.242
C14:2-OH	0.05 ± 0.00	0.06 ± 0.01	1.21[a]	0.06 ± 0.01	1.12	**0.008**
C10	0.18 ± 0.03	0.22 ± 0.06	1.25	0.20 ± 0.03	1.13	0.225
C16:1-OH	0.06 ± 0.00	0.07 ± 0.00	1.25[a]	0.07 ± 0.01	1.17[a]	**0.001**
C12:1	0.12 ± 0.01	0.16 ± 0.01	1.33[a]	0.15 ± 0.02	1.27[a]	**<0.001**
C12	0.15 ± 0.02	0.20 ± 0.02	1.33[a]	0.19 ± 0.03	1.27[a]	**0.004**
C5-DC (C6-OH)	0.14 ± 0.02	0.17 ± 0.02	1.23[a]	0.18 ± 0.03	1.28[a]	**0.010**
C14:1-OH	0.05 ± 0.01	0.07 ± 0.01	1.47[a]	0.07 ± 0.00	1.29[a]	**0.001**
C2	20.62 ± 3.57	28.82 ± 4.45	1.40[a]	26.77 ± 3.64	1.30[a]	**0.004**
C6 (4:1-DC)	0.16 ± 0.03	0.20 ± 0.02	1.29[a]	0.21 ± 0.03	1.32[a]	**0.004**
C16-OH	0.02 ± 0.00	0.03 ± 0.00	1.35[a]	0.03 ± 0.00	1.32[a]	**0.008**
C14:1	0.25 ± 0.02	0.34 ± 0.04	1.38[a]	0.33 ± 0.04	1.33[a]	**<0.001**
C16:2-OH	0.01 ± 0.00	0.02 ± 0.00	1.58[a]	0.02 ± 0.00	1.38[a]	**<0.001**
C5-M-DC	0.29 ± 0.04	0.38 ± 0.08	1.29[a]	0.42 ± 0.07	1.43[a]	**0.006**
C16	0.29 ± 0.15	0.43 ± 0.14	1.49	0.43 ± 0.14	1.46	0.163
C14	0.28 ± 0.12	0.41 ± 0.14	1.50	0.40 ± 0.11	1.47	0.122

	C	F12		F24		
Acylcarnitine	**µmol/l**	**µmol/l**	**FC**	**µmol/l**	**FC**	***p*-value**
C16:1	0.06 ± 0.03	0.10 ± 0.03	1.68[a]	0.09 ± 0.02	1.50	**0.024**
C14:2	0.05 ± 0.01	0.07 ± 0.01	1.51[a]	0.07 ± 0.01	1.52[a]	**0.001**
C18	0.07 ± 0.01	0.12 ± 0.02	1.65[a]	0.11 ± 0.01	1.52[a]	**<0.001**
C18-OH	0.05 ± 0.00	0.09 ± 0.01	1.62[a]	0.09 ± 0.01	1.64[a]	**<0.001**
C3-DC (C4-OH)	0.28 ± 0.09	0.48 ± 0.05	1.73[a]	0.54 ± 0.08	1.94[a]	**<0.001**
C16:2	0.02 ± 0.00	0.04 ± 0.01	2.16[a]	0.04 ± 0.00	2.05[a]	**<0.001**
C18:1	0.21 ± 0.06	0.56 ± 0.21	2.71[a]	0.43 ± 0.05	2.08[a]	**<0.001**
C18:2	0.06 ± 0.01	0.17 ± 0.04	2.64[a]	0.14 ± 0.02	2.29[a]	**<0.001**

Data is presented as mean ± SD. *P*-values obtained by one-way ANOVA adjusted with Benjamini-Hochberg correction.
[a]$p < 0.05$ indicates statistical significance to C group and was obtained by Tukey post-hoc analysis.
Abbreviations: C0, free carnitine; C2, acetylcarnitine; C3, propionylcarnitine; C3:1, propenoylcarnitine; C3-OH, OH-propionylcarnitine; C4, butyrylcarnitine; C4:1, butenylcarnitine; C4-OH, OH-butyrylcarnitine; C5, valerylcarnitine; C5:1, tiglylcarnitine; C5:1-DC, glutaconylcarnitine; C5-DC, glutarylcarnitine; C5-M-DC, methylglutarylcarnitine; C5-OH, OH-valerylcarnitine; C6, hecanoylcarnitine; C6:1, hexenoylcarnitine; C7-DC, pimelylcarnitine; C8, octanoylcarnitine; C9, nonaylcarnitine; C10, decanoylcarnitine; C10:1, decenoylcarnitine; C10:2, decadienylcarnitine; C12, dodecanoylcarnitine; C12:1, dodecenoylcarnitine; C12-DC, dodecanedioylcarnitine; C14, tetradecanoylcarnitine; C14:1, tetradecenoylcarnitine; C14:1-OH, OH-tetradecenoylcarnitine; C14:2, tetradecadienylcarnitine; C14:2-OH, OH-tetradecadienylcarnitine; C16, hexadecanoylcarnitine; C16:1, hexadecenoylcarnitine; C16:1-OH, OH-hexadecenoylcarnitine; C16:2, hexadecadienylcarnitine; C16:2-OH, OH-hexadecadienylcarnitine; C16-OH, OH-hexadecanoylcarnitine; C18, octadecanoylcarnitine; C18:1, octadecenoylcarnitine; C18:1-OH, OH-octadecenoylcarnitine; C18:2, octadecadienylcarnitine.

In mice, amino acids that were identified in the human trial as fasting markers (α-aminobutyrate and BCAA) were associated with changes in C0 and C2 levels as well, but not with C3 and C5 levels (Figure 25). Thus, fasting in the HuMet-study and in mice induced similar plasma changes with respect to amino acids and acylcarnitines.

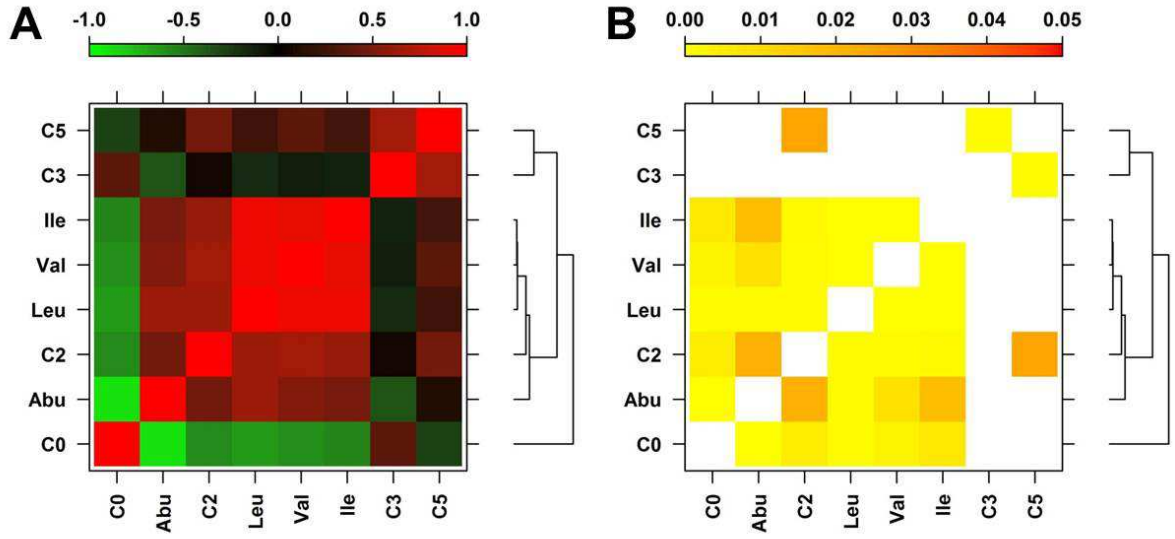


Figure 25: Correlation between selected acylcarnitine species, BCAA and α-aminobutyrate in mice during fasting
(A) Pearson correlation matrix between mean concentrations of selected acylcarnitine species, BCAA and α-aminobutyrate (B) and the corresponding matrix displaying the *p*-values of the correlation matrix.

Liver. In liver 37% of the measured amino acids were significantly altered in levels after fasting (Figure 26). The majority of these metabolites (asparagine, glutamine, serine and alanine, the aromatic amino acids tyrosine, phenylalanine, tryptophan, the branched-chain amino acids valine and isoleucine, as well as the total sum) declined in response to fasting. Only the concentrations of the derivatives α-aminobutyrate and β-aminoisobutyrate were higher in fasted animals compared to controls.

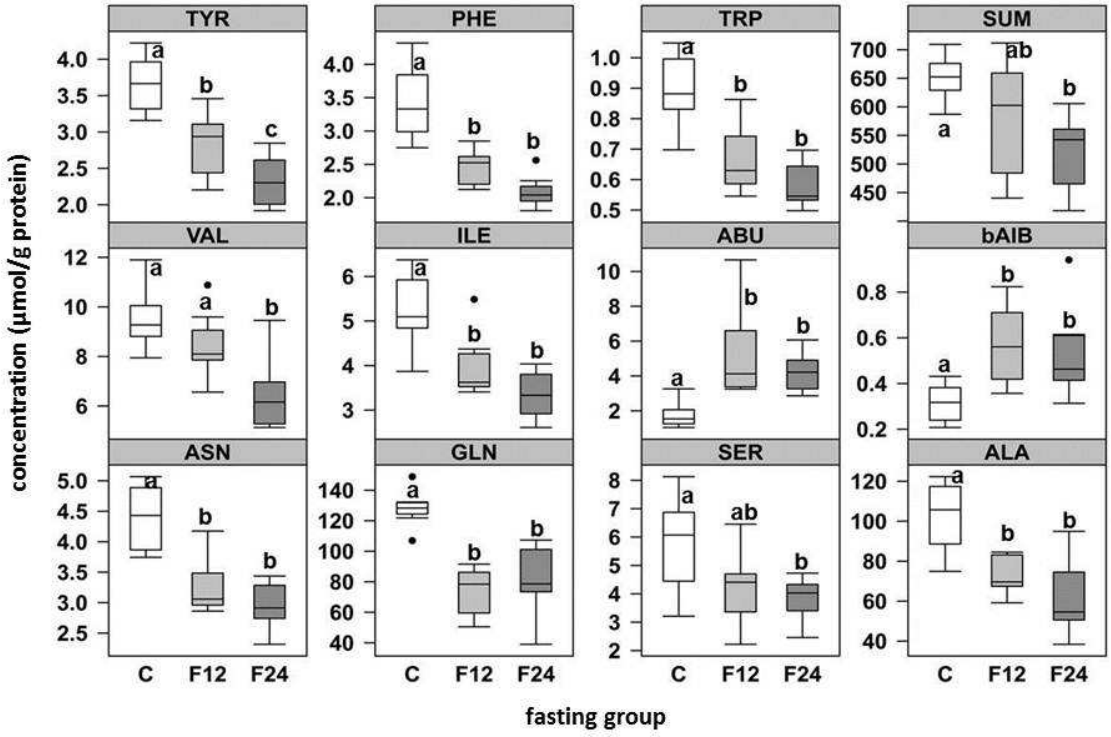


Figure 26: Significantly regulated liver amino acids in fasted mice, measured by LC-MS/MS

Mice were either fasted for 6 hours (C), 12 hours over night (F12) or 24 hours (F24). N=8 per group. Dots represent outliers. *P*-values obtained by one-way ANOVA adjusted with Benjamini-Hochberg correction. Different subscript letters indicate statistical significance ($p < 0.05$) based on Tukey post-hoc analysis to compare between groups.

Abbreviations: ABU, α-aminobutyrate; ALA, alanine; ASN, asparagine; bAIB, β-aminoisobutyrate; GLN, glutamine; ILE, isoleucine; PHE, phenylalanine; SER, serine; SUM, total sum; TRP, tryptophane; TYR, tyrosine; VAL, valine.

The flux of NEFA from the periphery to hepatic tissue is enhanced during food deprivation and we therefore analyzed hepatic fatty acid concentrations by a GC-MS approach. Eight of the 19 determined fatty acid were significantly altered (Table 6). Amongst them, C20:3n6 was the only fatty acid with reduced levels in liver of fasted animals compared to controls. All other altered fatty acids (C18:3n6, C20:1, C20:5n3, C17:0, C18:1n9, C22:6n3, C14:0) displayed higher concentrations in fasted animals compared to control animals.

Table 6: Hepatic fatty acid concentrations during fasting in mice
Mice were either fasted for 6 hours (C), 12 hours over-night (F12) or 24 hours (F24). N=7-8 per group. Data is ordered based on FC of F24.

	C	F12		F24		
Fatty acids	**µg/mg protein**	**µg/mg protein**	**FC**	**µg/mg protein**	**FC**	***p*-value**
C20:3n6	1.95 ± 0.59	1.03 ± 0.25	0.53[a]	0.96 ± 0.20	0.49[a]	**<0.001**
C20:0	0.25 ± 0.06	0.19 ± 0.04	0.77	0.20 ± 0.05	0.81	0.115
C24:0	0.21 ± 0.03	0.18 ± 0.07	0.85	0.19 ± 0.05	0.91	0.486
C24:1	0.26 ± 0.04	0.24 ± 0.06	0.94	0.25 ± 0.06	0.94	0.811
C22:0	0.21 ± 0.04	0.17 ± 0.07	0.80	0.21 ± 0.05	0.98	0.304
C20:4n6	27.49 ± 7.69	28.69 ± 5.25	1.04	28.24 ± 5.11	1,03	0.930
C18:0	17.86 ± 5.25	16.77 ± 2.90	0.94	18.60 ± 3.14	1.04	0.661
C22:1	0.06 ± 0.03	0.06 ± 0.02	0.95	0.07 ± 0.02	1.13	0.636
C20:2	0.36 ± 0.22	0.43 ± 0.08	1.20	0.42 ± 0.10	1.17	0.614
C16:0	49.56 ± 24.97	63.38 ± 16.06	1.28	63.17 ± 20.17	1.27	0.363
C18:3n6	0.36 ± 0.11	0.67 ± 0.21	1.83[a]	0.58 ± 0.30	1.6	**0.047**
C20:1	0.52 ± 0.22	0.77 ± 0.18	1.48	0.83 ± 0.30	1.60	**0.050**
C20:5n3	0.75 ± 0.34	1.10 ± 0.21	1.47	1.21 ± 0.37	1.62[a]	**0.027**
C17:0	0.34 ± 0.07	0.54 ± 0.06	1.60[a]	0.56 ± 0.17	1.65[a]	**0.002**
C18:1n9	92.68 ± 37.30	150.82 ± 48.88	1.63	154.17 ± 54.67	1.66	**0.042**
C16:1	4.24 ± 3.46	10.56 ± 7.51	2.49	7.39 ± 4.21	1.74	0.111
C18:2n6c	55.46 ± 34.32	84.98 ± 28.63	1.53	102.30 ± 39.93	1.84	0.057
C22:6n3	11.73 ± 3.57	22.38 ± 3.92	1.91[a]	24.41 ± 5.30	2.08[a]	**<0.001**
C14:0	0.31 ± 0.19	1.01 ± 0.60	3.28[a]	0.83 ± 0.41	2.69	**<0.018**

Data is presented as mean ± SD. *P*-values obtained by one-way ANOVA. [a]$p < 0.05$ indicates statistical significance to C group and was obtained by Tukey post-hoc analysis.

Skeletal muscle. In muscle tissue, 59% of the amino acids changed significantly by fasting based on one-way ANOVA (Figure 27). Food deprivation led to a reduction of lysine, arginine, serine, alanine, glycine, glutamine and the derivatives hydroxyproline, citrulline and sarcosine. Total sum of amino acids and threonine concentrations were only reduced in the F12 group, but not in animals of the F24 group. Other amino acids like the derivative 3-M-histidine, aspartate, tryptophan and proline were higher in fasted animals compared to controls. Similar to the changes observed in plasma of fasted mice as well as in the fasting challenge of the HuMet-study, concentrations of BCAA, α-aminobutyrate and phenylalanine increased in skeletal muscle of food deprived animals.

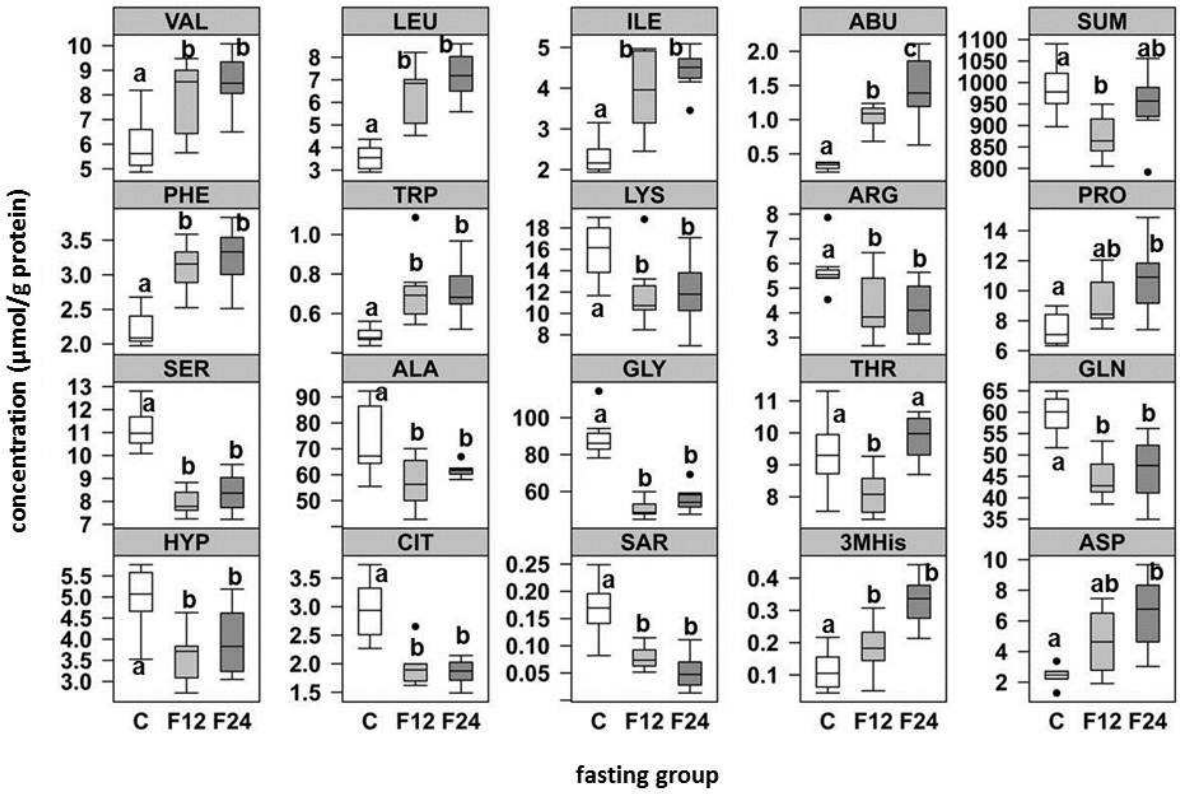
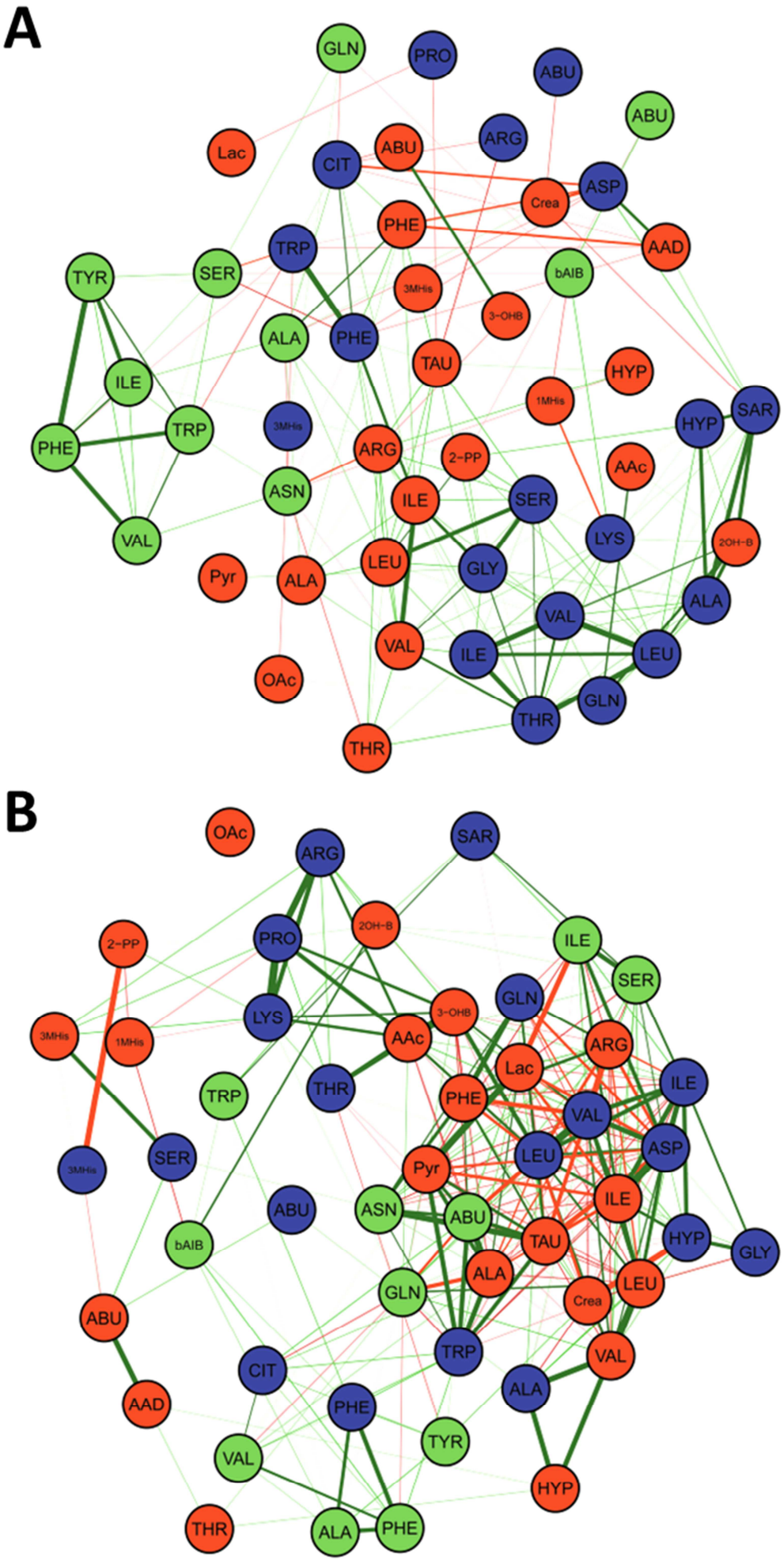


Figure 27: Significantly altered skeletal muscle amino acid levels in fasted mice, measured by LC-MS/MS

Mice were either fasted for 6 hours (C), 12 hours over night (F12) or 24 hours (F24). N=8 per group. Dots represent outliers. *P*-values obtained by one-way ANOVA followed by Tukey post-hoc analysis to compare between groups. Asterisks indicate statistical significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Abbreviations: 3MHis, 3-methyl-histidine; ABU, α-aminobutyrate; ALA, alanine; ARG, arginine; ASP, aspartate; CIT, citrulline; GLN, glutamine; GLY, glycine; HYP, hydroxyproline; ILE, isoleucine; LEU, leucine; LYS, lysine; PHE, phenylalanine; PRO, proline; SAR, sarcosine; SER, serine; SUM, total sum; THR, threonine; TRP, tryptophane; VAL, valine.

Correlation analysis. We identified in the human trial, as well as in the mouse study that in all compartments (plasma, liver and skeletal muscle) BCAA and α-aminobutyrate are most discriminating for the fasting condition. Previously, α-aminobutyrate was revealed as a fasting marker [82]. It is primarily a product of threonine metabolism and the alternative product of threonine catabolism is 2-OH-butyrate [66, 101], which was found to be associated with threonine and α-aminobutyrate [82]. Therefore we included the altered plasma metabolites measured via ^{1}H NMR when we submitted all regulated amino acids of plasma, liver and skeletal muscle to a correlation network (CN) analysis in order to visualize their interrelations (Figure 28A-C). All correlations r > 0.6 are represented by edges, while the width of the edges indicates the strength of the relation. Green colored edges indicate positive weights, while red edges are negative weights.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GLN
PRO
ABU
ABU
Lac
ABU
ARG
CIT
ASP
Crea
PHE
AAD
TRP
SER
TYR
3MHis
bAIB
3-OHB
ALA
PHE
TAU
HYP
ILE
1MHis
HYP
SAR
3MHis
TRP
PHE
ARG
2-PP
AAc
ASN
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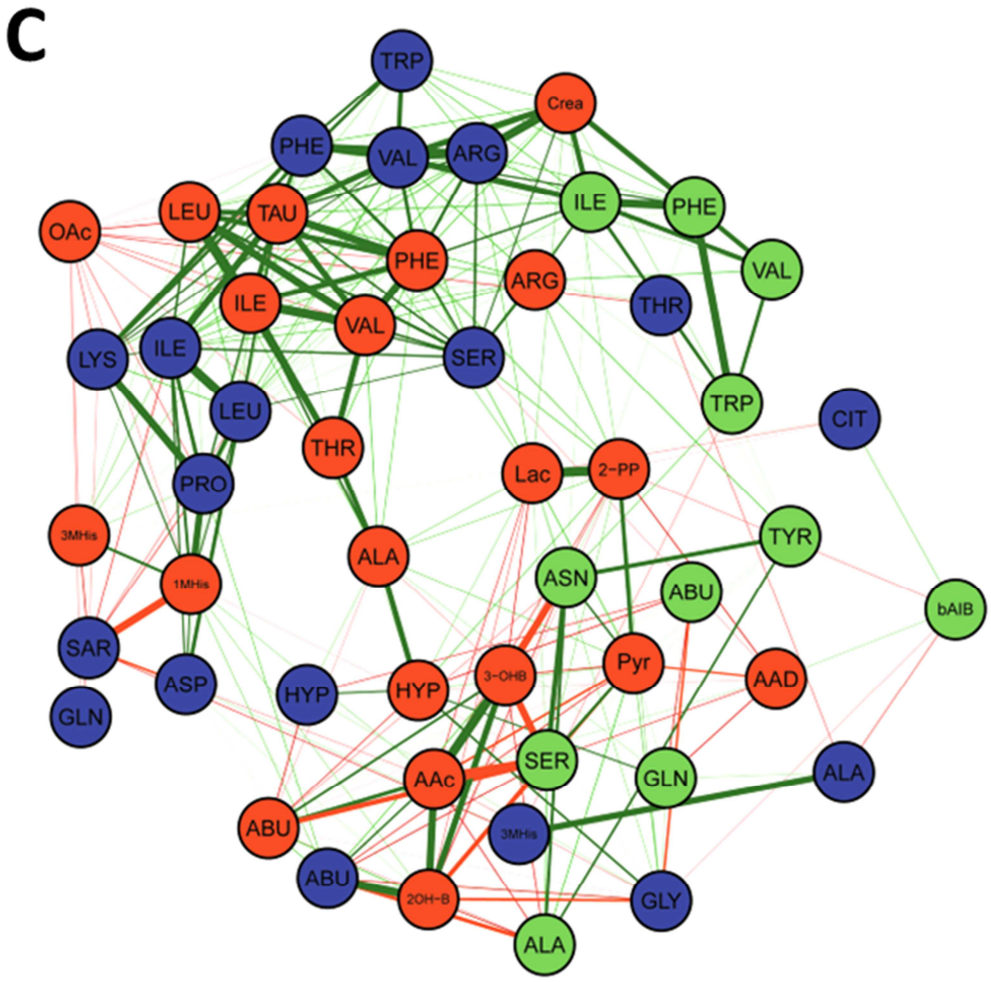

Figure 28: Correlation network analysis of regulated amino acids and metabolites measured by ^{1}H NMR in plasma, skeletal muscle and liver during fasting in mice

Each regulated metabolite is represented by a node. Red nodes represent plasma metabolites, green nodes liver metabolites and blue nodes metabolites measured in skeletal muscle. Nodes are connected by edges, when the correlation between both metabolites is r > 0.6. Length and width of the edge represent the strength of correlation and the cut-off was set to r = 0.8. Green colored edges indicate positive weights, while red edges are negative weights. (A) Correlation network of control time-point after a 6 hours fasting period in mice (n=8). (B) Correlation network after 12 hours of fasting in mice (n=7). (C) Correlation network after 24 hours of fasting in mice (n=7).
Abbreviations: See Figures 23, 26, 27; 2-OH-B, 2-OH-butyrate; 2-PP, 2-phenylpropionate; 3-OH-B, 3-OH-Butyrate; AAc, acetoaceta; Crea, creatine; Lac, lactate; Pyr, pyruvate; OAc, acetate.

The CN of control mice displayed only two small clusters and in both, BCAA of either liver or skeletal muscle are representative (Figure 28A). After a fasting period of 12 hours, there is one big cluster formed – mainly by plasma and skeletal muscle metabolites (Figure 28B). However, after 24 hours of fasting, there are two clusters formed which seemed to be connected via alanine measured in plasma (Figure 28C). Alanine is important for gluconeogenesis [86] and food deprivation reduced its concentrations in plasma, skeletal muscle and liver tissue in mice . Next to alanine, pyruvate and lactate are important precursors for *de novo* glucose production during

fasting and were highly associated to this amino acid (Figure 29AB). Interestingly after a fasting period of 24 hours, liver valine, leucine, phenylalanine and tryptophan are separated from other hepatic metabolites. Of note, plasma metabolites like acetoacetate, 2-OH-butyrate and 3-OH-butyrate cluster more to liver metabolites than to metabolites measured in skeletal muscle. Furthermore correlation over all fasting states revealed that regulated plasma metabolites like the BCAA, α-aminobutyrate, but also phenylalanine, 3-M-histidine and hydroxyproline were highly correlated ($r > 0.6$) to their corresponding muscle metabolites (valine: $r = 0.84$, leucine: $r = 0.89$, isoleucine: $r = 0.89$, α-aminobutyrate: $r = 0.91$, phenylalanine: $r = 0.75$, 3-M-histidine: $r = 0.74$, hydroxyproline: $r = 0.66$, for all $p < 0.001$). However, high correlations were only found between plasma α-aminobutyrate and alanine and their corresponding liver metabolite (α-aminobutyrate: $r=0.64$, $p < 0.01$; alanine: $r=0.7144$, $p < 0.001$). This indicates that the catabolic plasma metabolome mirrors the metabolite changes of skeletal muscle, but not of liver. This is also visible in the CN of the 24 hours fasting state, where there is a pronounced grouping for muscle and plasma metabolites. In contrast to the previous report of Rubio-Aliaga *et al*. we were not able to find an association between threonine and 2-OH-butyrate in plasma ($r = 0.23$, NS) when analysis included all time-points. However, plasma, liver and skeletal muscle concentrations of α-aminobutyrate were highly associated to plasma concentrations of 2-OH-butyrate (Figure 29C-E). For instance, plasma 2-OH-butyrate and α-aminobutyrate measured in skeletal muscle are also associated in the correlation network of the 24 hours fasting time-point (Figure 28C). In addition, there was an inverse correlation found between hepatic concentrations of threonine and α-aminobutyrate (Figure 29F).

An overview of all plasma metabolites of the fasting challenge in mice is given in Suppl. Table 27. The corresponding liver and skeletal muscle amino acids of the experiment are summarized in Suppl. Table 28 respectively Suppl. Table 29.

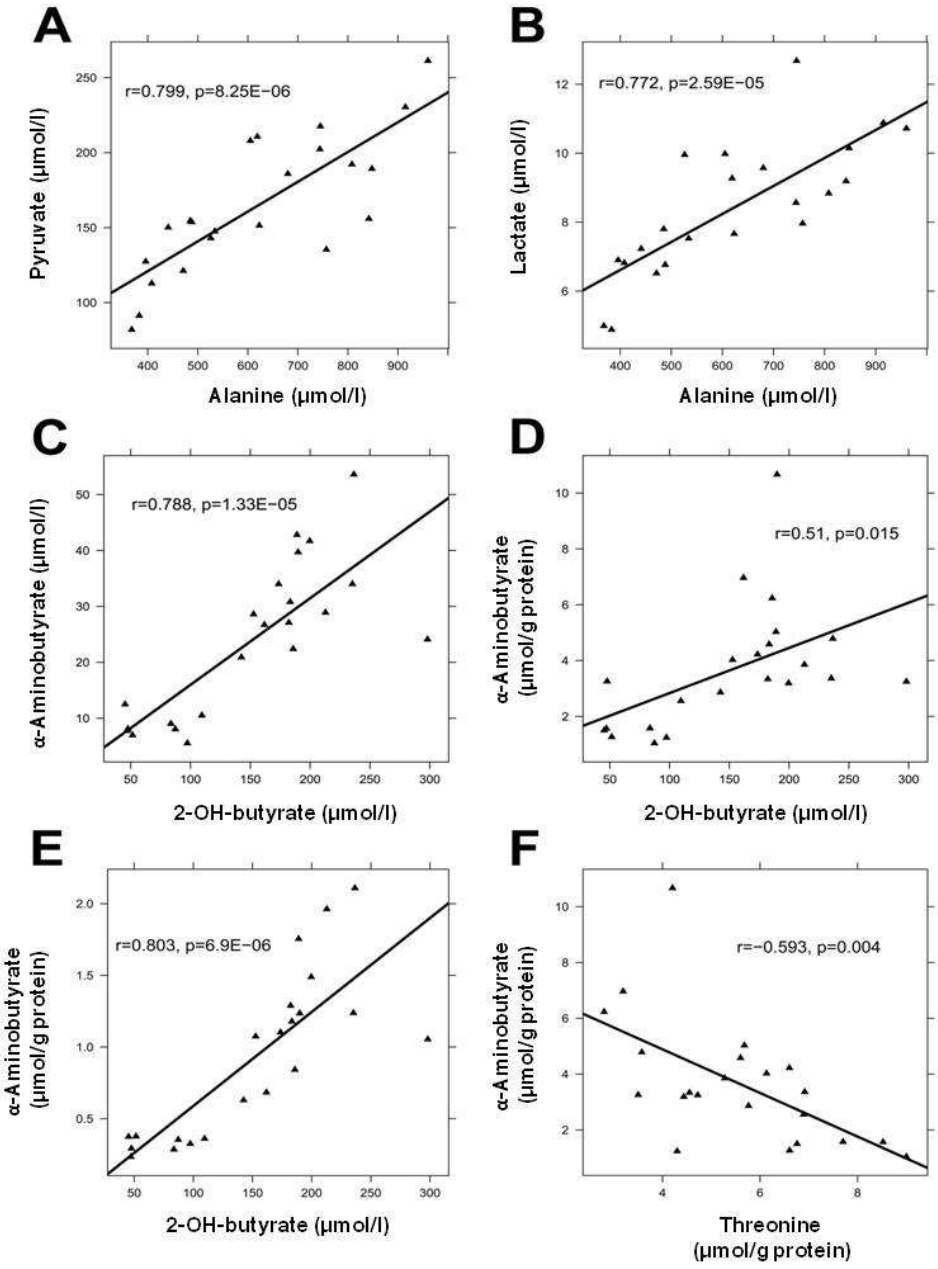

Figure 29: Pearson correlations of metabolites during fasting in mice
Pearson correlation between (A) alanine and pyruvate in plasma, (B) alanine and lactate in plasma. Pearson correlation between plasma 2-OH-butyrate and (C) plasma α-aminobutyrate, (D) liver α-aminobutyrate, and (E) α-aminobutyrate measured in skeletal muscle. (F) Pearson correlation between threonine and α-aminobutyrate measured in liver tissue. Each triangle represents 1 mouse.

In summarizing these findings in mice, it can be concluded that similar changes in plasma levels of metabolites occur in fasting conditions and after an oral glucose load. In addition to plasma levels, metabolites in organs such as liver and skeletal muscle show distinct alterations in both metabolic states that correlate with the systemic changes.

2.2. Metabolic changes in diet-induced obese mice (DIO mice)

Various human studies revealed that the metabolic syndrome is characterized by changes in plasma amino acids, mainly of BCAA [8, 11, 12, 15]. As shown above, C57BL/6 mice produce very similar changes in plasma metabolites under anabolic and catabolic conditions as humans. Although mouse models are frequently used to investigate human metabolic impairments linked to the metabolic syndrome, changes in the metabolome induced by dietary interventions in C57BL/6 mice are yet not well characterized. We therefore analyzed in DIO mice in three independent HF feeding trials the changes in metabolites with a focus on amino acids. Study 1 was embedded in the proof of principle study 3 (PPS3) of the nutrigenomics organization (NuGO) [102] and focused on the time-dependent changes in plasma during the development of the obese phenotype. In addition, effects of HF feeding on liver amino acids were examined after 12 weeks. In study 2, analysis of the amino acid metabolome was expanded to other tissues and concentration changes were assessed in plasma, liver, skeletal muscle, kidney and small intestine. By using an untargeted GC-MS approach, analysis of the diet-induced changes in plasma was extended to other metabolite groups in study 3.

2.2.1. Body and liver weight in mice fed a high-fat diet

Effects of HF dietary intervention were analyzed after 12 weeks. In study 1, mice were fed either a control diet (10 energy% (E%) of fat) or a diet containing 45 E% of fat. HF animals gained more weight compared to control animals (Figure 30A) and weight development showed a highly significant week x fat interaction based on two-way ANOVA ($p < 0.001$) (Figure 30B). Furthermore, liver weight was higher in HF animals compared to control animals (10 E%: 1.00 ± 0.02 g, 45 E%: 1.29 ± 0.02 g, $p < 0.001$). In study 2, dietary HF intervention led to an elevated body weight and liver weight in HF animals (60 E%) compared to animals fed a control diet (11 E%) (body weight: 11 E%: 32.14 ± 1.83 g vs. 60 E%: 42.48 ± 4.32 g, $p < 0.001$; liver weight: 11 E%: 1.40 ± 0.12 g vs. 60 E%: 1.83 ± 0.30 g, $p = 0.001$). The same findings were obtained in study 3. Mice that were fed a HF diet (48 E%) increased body weight significantly compared to control mice (12 E%) (12 E%: 27.65 ± 1.45 g vs. 48 E%: 35.16 ± 3.73 g, $p < 0.001$). Liver weight was not determined in study 3.

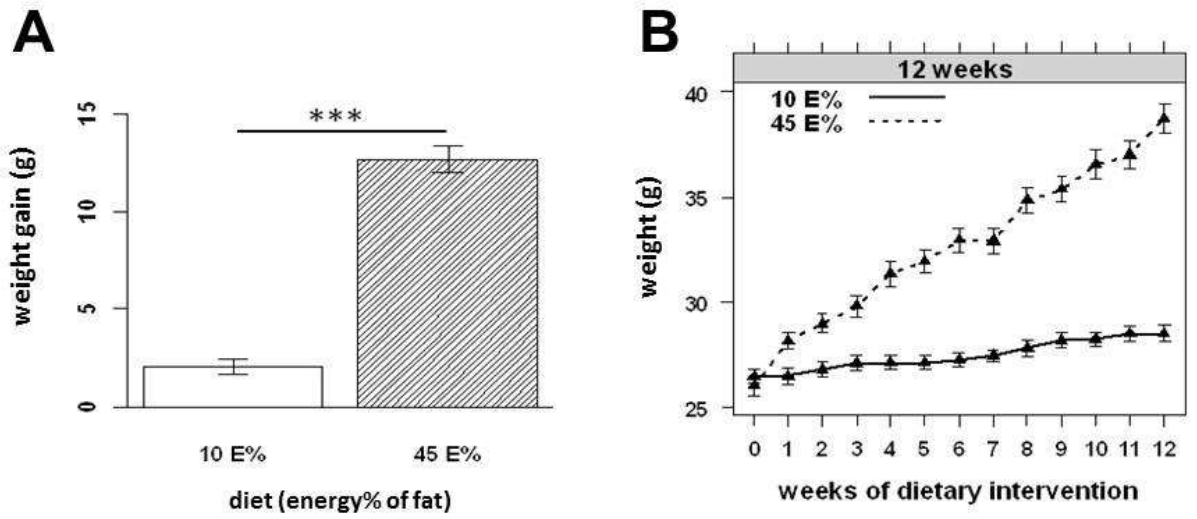

Figure 30: Body weight gain and body weight development over time in study 1
Weight gain of mice, that were fed either a control diet of 10 energy% of fat (10 E%) or a high-fat diet of 45 energy% of fat (45 E%) for 12 weeks (A). Weight development over the time-course of 12 weeks of high-fat feeding is shown. Solid lines represent animals on 10 E% fat diet, dotted lines represent animals on 45 E% fat diet (B). Data are presented as mean ± SEM. N=12 animals per group. ***$p < 0.001$ was obtained by unpaired t-test following Benjamini-Hochberg correction

2.2.2. Blood glucose in DIO mice

C57BL/6 mice are known to be susceptible to develop hyperglycemia when fed HF diets. In all HF-studies, we measured blood glucose levels after a defined fasting period of 5 or 6 hours. In study 1, mice were fasted for 5 hours and significant higher blood glucose concentrations were observed in mice fed a 45 E% fat diet compared to control mice (10 E%: 10.39 ± 1.05 mmol/l vs. 45 E%: 13.13 ± 0.65 mmol/l, $p < 0.001$) after 12 weeks of dietary intervention. In study 2, blood glucose concentrations were determined after 11 weeks of dietary intervention and a fasting period of 6 hours. Again, HF animals (60 E%) displayed significantly higher blood glucose levels than control animals (11 E%: 7.60 ± 0.70 mmol/l vs. 9.53 ± 0.86 mmol/l, $p < 0.001$). Glucose elevation in plasma of HF mice (48 E%) was also observed in study 3 (FC = 1.51, $p = 0.023$), when plasma glucose concentrations were assessed via an untargeted GC-MS approach in animals that were fasted for 6 hours.

2.2.3. Changes in the plasma metabolome of DIO mice

Study 1

In total, 30 amino acids and derivatives were quantified in plasma. For time-dependent changes during the 12 weeks of dietary HF intervention (45 E%), plasma amino acid concentrations were monitored every second week. Two-way ANOVA revealed, that only the two non-proteinogenic amino acids citrulline and hydroxyproline displayed a significant week x fat interaction (citrulline, $p < 0.05$; hydroxyproline $p < 0.05$) (Figure 31).

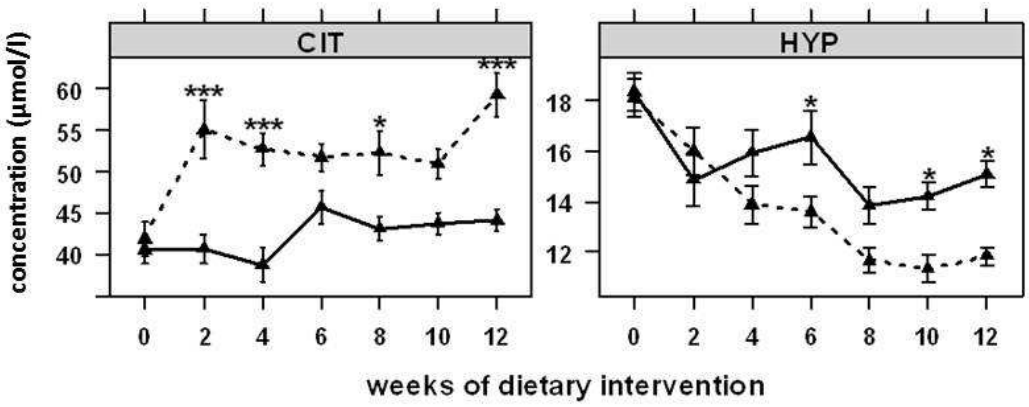

Figure 31: Time-course profiles of citrulline and hydroxyproline levels in plasma (study 1)
Solid and dotted lines represent control (n=12) and high-fat animals (n=12), respectively. Animals were fed either a 10 E% control diet or a 45 E% HF diet for 12 weeks. Data are presented as mean ± SEM. Statistical analysis was carried out by two-way ANOVA. Asterisks indicate statistical significance for week × fat-interaction based on Tukey post-hoc test. *$p < 0.05$, ***$p < 0.001$.
Abbreviations: CIT, citrulline; HYP, hydroxyproline.

Additional between-group comparison after week 12 was performed by using Student's t-test. In total, 8 amino acids were significantly altered. The two derivatives hydroxyproline and α-aminoadipate displayed decreased concentrations in DIO animals compared to controls (hydroxyproline: C: 15.08 ± 1.92 µmol/l vs. HF: 11.81 ± 1.35 µmol/l, p = 001; α-aminoadipate: C: 14.09 ± 2.69 µmol/l vs. HF: 11.39 µmol/l ± 1.75 µmol/l, p = 0.035). Citrulline, ethanolamine, glutamine, tyrosine and the urea-cycle intermediates arginine and ornithine were higher in HF animals (citrulline: C: 44.05 ± 4.59 µmol/l vs. HF: 59.23 ± 9.36 µmol/l, p = 0.001; ethanolamine: C: 12.42 ± 1.23 µmol/l vs. HF: 14.32 ± 1.75 µmol/l, p = 0.034; glutamine: C: 598.08 ± 26.76 µmol/l vs. HF: 676.8 ± 41.22 µmol/l, $p < 0.001$; tyrosine: C: 69.55 ± 10.71 µmol/l vs.

HF: 85.88 ± 16.71 µmol/l, p = 0.03; arginine: C: 76.68 ± 4.97 µmol/l vs. HF: 87.92 ± 12.02 µmol/l, p = 0.035; ornithine: C: 39.66 ± 3.32 µmol/l vs. HF: 49.33 ± 7.76, p = 0.005). Interestingly, in contrast to obese humans or type 2 diabetic patients, DIO mice did not display any significant alterations in plasma BCAA levels, neither for week × fat interaction (leucine, p = 0.833; isoleucine, p = 0.599; valine, p = 0.318), nor for between-group comparison after 12 weeks (leucine, C: 122.95 ± 17.23 vs. HF: 123.62 ± 13.01 µmol/l, p = 0.946; isoleucine, C: 82.12 ± 13.56 vs. HF: 83.08 ± 8.72 µmol/l, p = 0.946; valine, C: 191.75 ± 26.99 vs. HF: 199.42 ± 27.48 µmol/l, p = 0.617).

Study 2

In HF animals (60 E%) diminished plasma concentrations were found for α-aminoadipate (p < 0.05; Figure 32A) and elevated concentrations were found for asparagine, ornithine, serine and 3-M-histidine (p < 0.05; Figure 32B-E) when compared to control animals (11 E%).

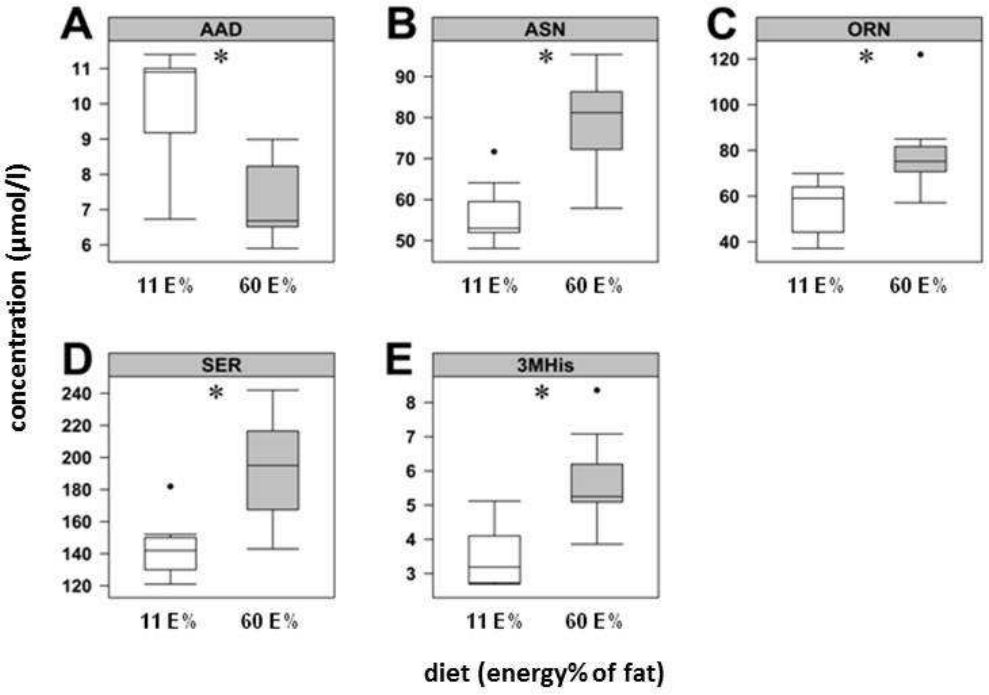

Figure 32: Significantly altered plasma amino acid levels in study 2
Effect of 12 weeks 60 energy% high-fat feeding on plasma amino acids. Plasma profiles of regulated amino acids are displayed. (A) AAD (α-aminoadipate), (B) ASN (asparagine), (C) ORN (ornithine), (D) SER (serine) and (E) 3MHis (3-methyl-histidine). Open and grey boxes represent control (n=9) and high-fat animals (n=7). (•) indicate outliers. *P*-values were obtained by unpaired t-test and post-hoc analysis using Benjamini-Hochberg correction. Asterisks indicate statistical significance. *p < 0.05.

Like in study 1, concentrations of BCAA remained unchanged (leucine, C: 222.44 ± 37.79 µmol/l vs. HF: 203 ± 43 µmol/l, p = 0.493; isoleucine, C: 138.0 ± 23.43 µmol/l vs. HF: 130.0 ± 19.39 µmol/l, p = 0.599; valine, C: 346.0 ± 56.85 µmol/l vs. HF: 312.86 ± 37.35 µmol/l, p = 0.318) and citrulline concentrations were elevated in HF animals compared to controls (C: 48.41 ± 9.11 µmol/l vs. HF: 63.31 ± 9.49 µmol/l, p = 0.088).

Study 3

In study 3, an untargeted GC-MS approach was applied to analyze the plasma changes after HF feeding. In total 118 metabolites were identified, but only 13 of these metabolites (11%) changed significantly in DIO mice (Table 7).

Table 7: Regulated plasma metabolites obtained by GC-MS approach (study 3)
Mice were fed either a control diet (12 energy% of fat, n=6) or a high-fat diet (48 energy% of fat, n=5). All metabolites with a p-value < 0.05 are displayed and ordered by fold-change (FC).

Metabolite (normalized peak intensity)	12 E%	48 E%	FC	p-value
9,12-Octadecadienoic acid (Z,Z)	727.16 ± 118.51	561.31 ± 53.84	0.77	0.018
Fatty acid (MW 3726.67)	567.14 ± 203.14	1075.56 ± 235.68	1.20	0.004
Hexadecanoic acid (C16:0)	1684.11 ± 149.42	2080.34 ± 145.06	1.24	0.002
2-oxo-gluconic acid	3672.25 ± 4153.69	11686.62 ± 4028.06	1.34	0.010
α-Tocopherol	298.39 ± 80.57	417.93 ± 93.50	1.40	0.049
Glucose	73019.83 ± 27237.25	110146.49 ± 14409.74	1.51	0.023
Glucaric acid-1,4-lactone	2565.21 ± 905.37	3874.25 ± 656.41	1.51	0.025
Cholesterol	6895.07 ± 2204.96	11034.02 ± 1683.64	1.60	0.007
Cholesterol (derivate)	55.84 ± 22.96	95.09 ± 26.57	1.70	0.027
Sterol	7.49 ± 1.37	12.91 ± 4.08	1.72	0.013
Hexose	1718.88 ± 559.03	3186.64 ± 703.95	1.85	0.004
Sugar alcohol	130.42 ± 75.14	244.31 ± 56.80	1.87	0.021
Fatty acid (MW 3741.38)	49.09 ± 28.28	129.70 ± 40.53	2.64	0.004

Data are presented as mean ± SD. P-value obtained by unpaired t-test.

The fatty acid 9,12-(Z,Z)-octadecadienoic acid displayed lower plasma concentrations in HF mice (48 E%) compared to control animals (12 E%). All other metabolites were increased in DIO animals (hexadecanoic acid, two fatty acid derivatives, hexose, cholesterol and its derivate, 2-oxo-gluconic acid, sterol, sugar alcohol, glucose (see 2.2.2), glucaric acid-1,4-lactone, and α-tocopherol). Between-group comparison again revealed, that plasma BCAA concentrations were not changed by the HF intervention (valine: C: 1715.43 ± 516.55 normalized peak

intensity (npi) vs. 1713.53 ± 135.77 npi, p = 0.994; leucine: C: 509.38 ± 333.49 npi vs. HF: 574.54 ± 97.27 npi, p = 0.685; isoleucine: C: 212.48 ± 113.49 npi vs. HF: 247.17 ± 75.37 npi, p = 0.575).
Data of study 1 are summarized in Suppl. Table 30 and amino acid levels obtained in study 2 are summarized in Suppl. Table 31. All identified metabolites of the GC-MS approach (study 3) are summarized in Suppl. Table 32.

2.2.4. Alterations in citrulline and arginine metabolism

Since study 1 and study 2 revealed increased plasma citrulline concentrations in obese animals with a significant week x fat interaction over 12 weeks (see 2.2.3., Figure 31) we next focused on the possible origin of that. Citrulline has a distinct inter-organ cycle which involves small intestine and kidney [39] and is also an intermediate of the urea cycle in liver [39]. Embedded into study 1 was a transcriptome profiling for the short-term effects of 4 weeks HF intervention that has been already published [103]. This analysis revealed that the expression of carbamoyl-phosphate synthetase 1 and arginase type II increased in mRNA levels significantly (2.2-fold respectively 1.24-fold) by HF feeding.

In particular, the increase in arginase type II suggested an enhanced conversion rate from arginine to citrulline via the intermediate ornithine. Thus, we hypothesized that plasma citrulline concentrations may increase in DIO due to an enhanced intestinal citrulline production in HF animals. The everted gut sacs technique with jejunal tissues of obese mice in combination with $^{13}C^{15}N$-labeled arginine was used in order to assess the intestinal release of citrulline from stable-isotope labeled arginine via the intermediate ornithine. This approach may also reflect the intestinal arginase activity (for more detail of the pathway see 4.7 and Figure 37). The conversion rate was quantified by the application of LC-MS/MS analysis. Surprisingly, the arginine to citrulline conversion calculated as the release of citrulline (Figure 33A) respectively ornithine (Figure 33B) per mg intestinal protein remained unchanged in tissues of DIO mice as compared to controls.

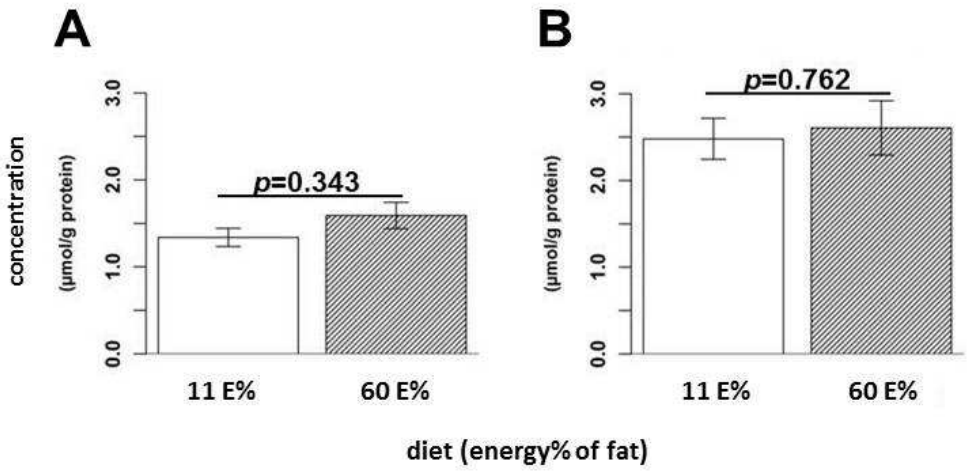

Figure 33: Intestinal release of citrulline (A) and ornithine (B) from $^{13}C^{15}N$-arginine in intestinal segments *in vitro*

Concentrations are standardized by intestinal protein concentrations. Data are presented as mean ± SEM. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction.

Amino acid concentrations in the small intestine and kidney were also measured, as both tissues are involved in the inter-organ citrulline-arginine cycle. At the end of the feeding trial, concentrations of the urea cycle intermediates citrulline, ornithine, arginine and argininosuccinate in the small intestine as well as in renal tissue remained unchanged (small intestine: citrulline: 11 E%: 5.77 ± 0.81 µmol/g protein vs. 60 E%: 6.97 ± 1.81 µmol/g protein, p = 0.856; ornithine: 11 E%: 19.06 ± 2.61 µmol/g protein vs. 60 E%: 20.76 ± 4.05 µmol/g protein, p = 0.856; arginine: 11 E%: 89.73 ± 21.39 µmol/g protein vs. 60 E%: 89.2 ± 25.29 µmol/g protein, p = 0.999; argininosuccinate: 11 E%: 4.17 ± 1.45 µmol/g protein vs. 60 E%: 2.53 ± 1.14 µmol/g protein, p = 0.77; kidney: citrulline: 11 E%: 0.48 ± 0.13 µmol/g protein vs. 60 E%: 0.55 ± 0.09 µmol/g protein, p = 0.427; ornithine: 11 E%: 1.46 µmol/g protein vs. 60 E%: 1.57 ± 0.46, p = 0.49; arginine: 11 E%: 4.31 ± 0.82 µmol/g protein vs. 60 E%: 4.01 ± 0.64 µmol/g protein, p = 0.564; argininosuccinate: not detectable). In kidney, HF feeding led to a reduction of the carnitine concentrations in HF animals compared to controls (C: 0.42 ± 0.13 µmol/g protein vs. HF: 0.22 ± 0.04 µmol/g protein, p = 0.03). Amino acid data of kidney and small intestine are summarized in Suppl. Table 33 respectively Suppl. Table 34.

Dietary arginine is the main precursor of citrulline in mice [45, 46]. Erdely *et al.* defined arginine bioavailability as the ratio of arginine to ornithine + citrulline [104]. In study 1 and study 2, we found a diminished plasma arginine bioavailability in HF animals (Table 8). Furthermore, arginine bioavailability was reduced in skeletal

muscle of DIO mice, but not in kidney and small intestine (Table 8). It is therefore unlikely that the elevated plasma citrulline concentrations originate from these two organs.

Table 8: Arginine bioavailability in plasma and tissues
Arginine bioavailability was calculated via Arginine/(Ornithine+Citrulline) in plasma and tissue of study 1 and study 2 after 12 weeks of high-fat dietary intervention.

Feeding trial		Control	High-fat	*p*-value
Study 1	**Plasma**	0.92 ± 0.08	0.81 ± 0.05	0.005
Study 2	**Plasma**	0.92 ± 0.13	0.62 ± 0.12	0.013
	Small intestine	3.59 ± 0.72	3.21 ± 0.56	0.856
	Skeletal muscle	1.43 ± 0.25	0.99 ± 0.15	0.006
	Kidney	2.38 ± 0.62	1.79 ± 0.41	0.27

Data are presented as mean ± SD. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction.

2.2.5. Alterations in hepatic metabolism in DIO mice

Hepatic citrulline is an intermediate of the urea cycle and we therefore determined hepatic amino acid concentrations after 12 weeks of HF feeding as well. In total 34 amino acids and derivatives could be quantified. Arginine concentrations in liver were generally below detection limit. In study 1 and study 2, 50% and 34% of the amino acids displayed significant concentration changes, respectively (Table 9). In both studies, hepatic concentrations of ornithine, glycine, serine, leucine, lysine, hydroxyproline and β-aminoisobutyrate were lower in obese animals compared to controls. In addition, HF animals of study 1 displayed elevated concentrations of glutamine and sarcosine, while levels of γ-aminobutyrate, β-alanine, α-aminoadipate, methionine, tyrosine, 1-M-histidine, P-ethanolamine and proline were reduced. In study 2, diminished concentrations were also found for valine, isoleucine, asparagine, phenylalanine, tryptophan, homocitrulline and α-aminobutyrate. Noteworthy, in both studies citrulline concentrations remained unchanged (study 1: 10 E%: 0.43 ± 0.15 µmol/g protein vs. 45 E%: 0.56 ± 0.17 µmol/g protein, p = 0.086; study 2: 11 E%: 0.42 ± 0.11 µmol/g protein vs. 60 E%: 0.36 ± 0.04 µmol/g protein, p = 0.197).

Table 9: Significantly regulated levels of amino acids in liver of DIO and control mice
Data is based on direction of change and p-value. In study 1, mice were either fed a control diet (10 E% of fat) or a high-fat diet (45 E% of fat). N=12 per group. In study 2, mice were either fed a control diet (11 E% of fat) or a high-fat diet (60 E% of fat). N=9 for 11 E% and n=8 for 60 E%.

Study 1.				
Amino acid (µmol/g protein)	**Change**	**10 E%**	**45 E%**	***p*-value**
Glutamine	↑	90.42 ± 9.55	106.17 ± 13.03	0.015
Sarcosine	↑	0.33 ± 0.10	0.51 ± 0.18	0.029
β-aminoisobutyrate	↓	0.45 ± 0.10	0.30 ± 0.04	0.003
Hydroxyproline	↓	0.53 ± 0.10	0.36 ± 0.09	0.004
γ-aminobutyrate	↓	0.50 ± 0.08	0.38 ± 0.06	0.005
β-Alanine	↓	2.24 ± 0.87	1.17 ± 0.33	0.006
Glycine	↓	62.24 ± 4.67	55.32 ± 4.77	0.012
Ornithine	↓	4.98 ± 0.87	3.68 ± 1.03	0.015
α-aminoadipate	↓	3.58 ± 1.99	1.76 ± 0.63	0.025
Lysine	↓	12.37 ± 1.59	10.37 ± 1.62	0.025
Serine	↓	10.91 ± 3.17	7.64 ± 2.27	0.029
Methionine	↓	1.74 ± 0.51	1.22 ± 0.37	0.030
Tyrosine	↓	5.24 ± 0.87	4.29 ± 0.84	0.035
Leucine	↓	10.70 ± 2.08	8.69 ± 1.69	0.041
1-M-histidine	↓	0.71 ± 0.12	0.61 ± 0.07	0.043
P-Ethanolamine	↓	7.68 ± 1.35	6.47 ± 1.06	0.048
Proline	↓	5.98 ± 0.76	5.23 ± 0.73	0.048
Study 2.				
Amino acid (µmol/g protein)	**Change**	**11 E%**	**60 E%**	***p*-value**
β-aminoisobutyrate	↓	0.15 ± 0.04	0.08 ± 0.02	0.009
Serine	↓	7.53 ± 0.79	5.85 ± 0.83	0.012
Valine	↓	12.72 ± 1.61	9.56 ± 1.57	0.012
Glycine	↓	48.20 ± 6.91	37.94 ± 3.42	0.014
Homocitrulline	↓	0.11 ± 0.04	0.05 ± 0.03	0.014
Lysine	↓	12.32 ± 1.51	9.90 ± 1.16	0.014
Tryptophan	↓	1.16 ± 0.12	0.95 ± 0.12	0.014
Ornithine	↓	6.18 ± 1.20	4.55 ± 0.62	0.016
α-aminobutyrate	↓	0.96 ± 0.37	0.50 ± 0.12	0.018
Isoleucine	↓	6.55 ± 0.93	5.08 ± 0.92	0.018
Asparagine	↓	4.65 ± 0.46	3.91 ± 0.51	0.022
Hydroxyproline	↓	0.50 ± 0.10	0.38 ± 0.06	0.033
Leucine	↓	11.63 ± 1.94	9.22 ± 1.46	0.033
Phenylalanine	↓	4.21 ± 0.48	3.52 ± 0.56	0.039

Data are presented as mean ± SD. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction.

Both urea cycle intermediates ornithine and citrulline share the ornithine/citrulline exchange system and correlation analysis revealed an association between the concentrations of hepatic ornithine and citrulline measured in plasma (Figure 34).

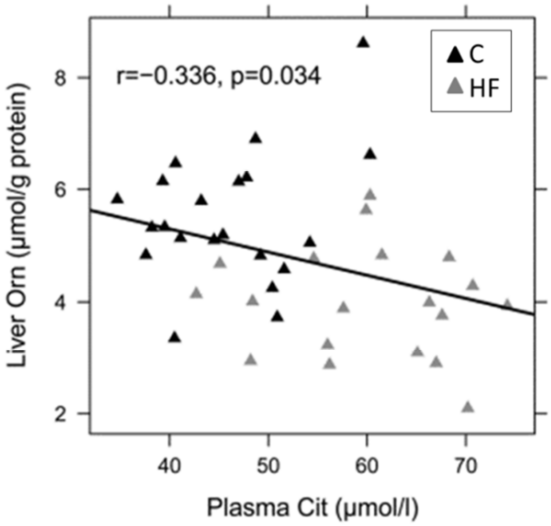

Figure 34: Correlation between liver ornithine concentration and plasma citrulline concentration
Each triangle represents one mouse of either study 1 or study 2. Black triangles are control mice (10 E% or 11 E%), grey triangles are high-fat mice (45 E% or 60 E%),
Abbreviations: Cit, citrulline; C, control; HF, high-fat; Orn, ornithine.

When mice and rats are fed a HF diet, hepatic glucose production is enhanced [105, 106]. Glucogenic amino acids can serve as substrates for endogenous glucose production. In both studies, total sum and the sum of glucogenic amino acids (GAA) remained unchanged in DIO mice (study 1: sum: C: 610.98 ± 54.02 µmol/g protein vs. HF: 646.59 ± 56.05 µmol/g protein, p = 0.164; GAA: C: 352.75 ± 33.14 µmol/g protein vs. HF: 358.32 ± 33.06 µmol/g protein, p = 0.67; study 2: sum: C: 554.21 ± 67.85 µmol/g protein vs. HF: 560.67 ± 41.59 µmol/g protein, p = 0.881; GAA: C: 323.68 ± 45.98 µmol/g protein vs. HF: 318.03 ± 27.27 µmol/g protein, p = 0.766). However, since glutamine (Gln) and alanine (Ala) are far higher than those of the other GAA, we also determined the sum for GAA when omitting glutamine and alanine GAA-(Ala+Gln). This revealed in DIO animals significantly lower concentrations (study 1: C: 168.40 ± 16.70 µmol/g protein vs. 149.22 ± 15.06 µmol/g protein, p = 0.164; study 2: C: 150.44 ± 20.76 µmol/g protein vs. 127.12 ± 7.90 µmol/g protein, p = 0.013) and suggested that the enhanced gluconeogenesis in DIO mice is associated with changes in these amino acids. When *de novo* glucose production from amino acids is increased, nitrogen detoxification via the urea cycle

has to be enhanced. Therefore, we correlated GAA-(Ala+Gln) with the urea cycle intermediate ornithine. Interestingly, for both studies there was a high correlation found between liver ornithine concentrations and GAA-(Ala+Gln) concentrations (study 1, $p < 0.001$; Figure 35A, study 2, $p < 0.001$; Figure 35B). Hepatic amino acid data of study 1 and study 2 are summarized in Suppl. Table 37 and Suppl. Table 37.

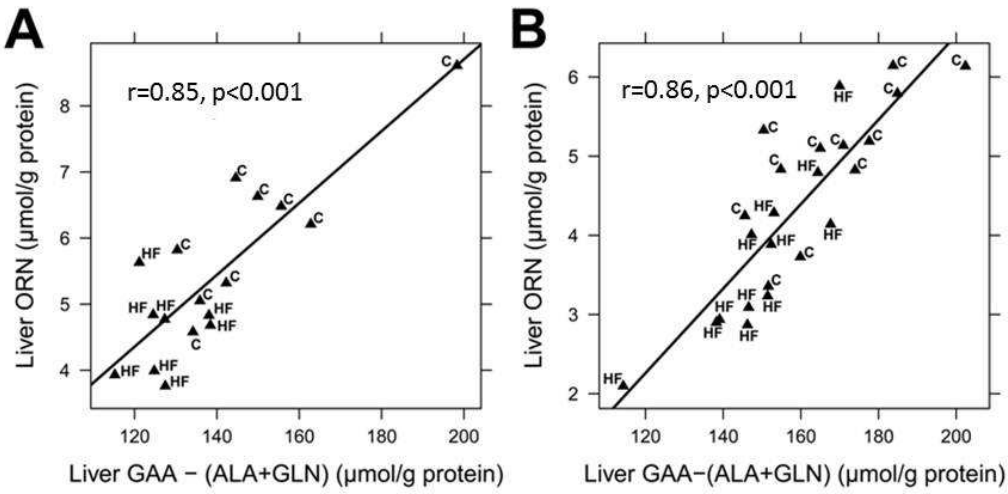

Figure 35: Correlation between liver ornithine concentrations and liver 'GAA-(Ala+Gln)'

Mice were fed either a control or a high-fat diet for 12 weeks. (A) study 1 (C: 10 energy% fat, n=12; HF: 45 energy% fat, n=12), (B) study 2 (C: 11 energy% fat, n=9; HF: 60 energy% fat, n=8). Each triangle represents 1 mouse.

Abbreviations: GAA, glucogenic AA; ALA, alanine; GLN, glutamine; C, Control group; HF, High-fat group.

2.2.6. Amino acid changes in skeletal muscle

In skeletal muscle, DIO animals (60 E%) displayed elevated concentrations of asparagine and serine (asparagine: C: 2.73 ± 0.23 µmol/g protein vs. HF 3.75 ± 0.52 µmol/g protein, $p < 0.01$; serine: C: 9.82 ± 1.29 µmol/g protein vs. HF: 12.37 ± 1.97 µmol/g protein; respectively, $p < 0.01$) and reduced concentrations of lysine and the derivatives carnosine and sarcosine (lysine: C: 19.66 ± 4.27 µmol/g protein vs. HF: 13.47 ± 2.64 µmol/g protein, $p < 0.05$; carnosine: HF: 70.41 ± 7.55 µmol/g protein vs. HF: 56.24 ± 4.77 µmol/g protein, $p < 0.01$; sarcosine: HF 0.17 ± 0.02 µmol/g protein vs. HF: 0.1 ± 0.02 µmol/g protein; $p < 0.001$). In addition, concentrations of essential AA and ketogenic AA were diminished in obese animals compared to controls (essential AA: C: 52.65 ± 5.19 µmol/g protein vs. 46.4 ± 5.03 µmol/g protein, $p < 0.05$; ketogenic AA: C: 24.24 ± 4.32 µmol/g protein vs. HF 17.88 ± 2.67 µmol/g protein, $p < 0.05$). Skeletal muscle amino acid data are summarized in Suppl. Table 37.

3. Discussion

Metabolite profiling techniques are widely used in various areas of the life and biomedical sciences. They are also employed as discovery tools for diagnostic and or prognostic markers for several diseases [5-7]. Such prognostic biomarkers should allow the identification of an individuals' risk to develop a disease in early stage. However, this requires the detection of rather subtle changes already in "pre-disease" conditions and this on background of a profound metabolic variability in bio fluids (i.e. plasma and urine). Just recently, Wang *et al.* proposed that BCAA, tyrosine and phenylalanine in plasma are potential prognostic markers for the development of T2DM [12]. Despite intensive biochemical and nutritional research in the last decades, amino acid metabolism in healthy and disease conditions is still not fully understood. The current knowledge does not allow to define the origin of the changes in amino acid biomarkers in insulin-resistant humans or type 2 diabetics. It was the goal of the present work to study the systemic changes in amino acid pools in healthy humans undergoing different metabolic challenges in order to reveal the dynamics of alterations. Furthermore, this was extended to mouse studies that may help to define the disease-related causes by allowing also studying the changes in organ metabolomes.

3.1. The amino acid metabolome in various metabolic conditions of healthy humans

3.1.1. The baseline metabolome (over-night fasting samples)

In the HuMet-study, 50 blood and 15 urine samples including 3 baseline and over-night fasting samples (day 1, day 3 and day 4), were collected from each of the 15 healthy volunteers over 4 days and amino acids were analyzed. For the baseline samples, repeated-measurement ANOVA revealed a fairly stable and reproducible pattern. Previous studies have also reported a remarkable stability of the metabolome. For example, in plasma samples only small inter-subject variabilities were found [79, 80]. In a human trial, a dietary standardization prior to metabolome analysis lowered only the variability of the first-void urine, but not that of plasma [80]. Winnike and coworkers reported that a normalization of the serum metabolome was

achieved for both, lipid components and glucose levels by one day of dietary standardization in healthy volunteers [81]. The apparent stability of the fasting metabolome over repeated measures and independent days in the HuMet-study confirms that the preceding food intake has only minimal effects of over-night fasting plasma samples. However, basal amino acid levels changed slightly on day 4. These changes seems to originate from the exercise test on day 3, which obviously appears to influence the plasma and urinary amino acid levels up to the next morning. It is beyond the scope of the current work, to answer what causes the identified concentration changes in plasma and urine after the CET, but for instance elevated phenylalanine concentrations and reduced ornithine concentrations in plasma were previously linked to physical activity [88, 107, 108].

We further assessed between- and within-subject variability of the baseline samples and found similar to an in-house study (Caroline Heim, Molecular Nutrition Unit, Technische Universität München, unpublished data) a lower variability for plasma samples as compared to urine samples. Plasma 3-M-histidine levels displayed highest between-subject variability. 3-M-histidine measured in urine is associated to fat-free mass and therefore considered as indicator for total muscle mass [109]. However, in the present study [98] no association between muscle mass of the volunteers (measured by dual energy X-ray absorptiometry) and plasma 3-M-histidine concentrations in baseline samples was found. In contrast to plasma in which 3-M-histidine showed highest variability, urine samples displayed between-subject variabilities with a CV > 0.5 for 4 amino acids and predominantly for lysine. Interestingly, histidine was amongst the amino acids with high between-subject variability as well. The question of why those amino acids are revealing a high variability in urinary output cannot be answered yet. However, it may be speculated that renal amino acid transporters and genetic heterogeneity may play a role. Lysine and histidine are reabsorbed in kidney by the sodium-independent transporter encoded by the SLC7A9 gene. A recent genome-wide association study identified a single-nucleotide polymorphism rs8101881 in the SLC7A9 gene and this could be linked to metabolite ratios including urinary lysine concentrations [110] suggesting that the polymorphism influences renal lysine handling.

3.1.2. Metabotype-specific signatures in the anabolic state

Over the 4 days, 5 defined metabolic challenges (fasting, CET, OGTT, OLLT, SLD) in the volunteers were applied, which consequently displaced the plasma and urine amino acid metabolome from its “steady state”. Time-course profiles of selected amino acids revealed the metabolome as an ever-changing continuum. All interventions, whether catabolic (fasting, CET) or anabolic (OGTT, SLD, OLTT) caused substantial changes in the amino acid profiles. Although we aimed to recruit a homogeneous group of volunteers (with respect to sex, BMI, age, *etc.*) to the study, the variability of amino acids depended on various aspects. The variability was specific to the measured amino acid itself suggesting that some amino acids underlie a more tight regulation by hormones and metabolic conditions than others. By nature, low abundant amino acids also display larger variation in plasma and urine concentrations than those with high concentrations. Every challenge per se increased the variability by distinctly different time-concentration profiles and PCA analysis revealed a metabotype specific amino acid metabolome in all anabolic challenges (OGTT, SLD, OLTT). Such a volunteer-specific metabolite signature was also reported for urine samples with repeated measures [111]. In addition, in response to a test meal, a volunteer specific signature was found as well [97]. Surprisingly amongst the catabolic challenges, only the CET revealed an individual fingerprint when PCA was performed with the amino acid data. However, when Krug and coworkers performed PCA analysis with data including compounds derived from β-oxidation such as the various acylcarnitine species including all time-points, a metabotype-specific grouping was observed. Similarly, also fasting produced the metabotype-specific grouping when lipids and in particular acylcarnitines were included [98]. At the current state, the origins of these individual responses that identify a person by its profile are unknown. In addition to genetic heterogeneity, numerous life style factors but also body constitution (muscle or fat mass) may represent crucial determinants.

3.1.3. 1-M-histidine as exposure marker for meat consumption

One of the most intriguing finding relates to urinary and plasma 1-M-histidine concentrations. Regardless of any challenge, the time-course profiles revealed a constant decline over the entire study. 1-M-histidine originates from anserine metabolism. Recent publications proposed urinary 1-M-histidine and 3-M-histidine

concentrations as exposure markers of meat consumption [112, 113]. As the HuMet-volunteers received solely standardized nutrient solutions over the 4 days of study, the decline of plasma and urine concentrations of 1-M-histidine over time presumably represents its elimination rate from the body. Noteworthy, there was no difference observed with respect to the absolute concentrations at the beginning of each 2 days block. All volunteers received a standardized dinner prior to the two test days that included chicken. Chicken-derived protein intake was previously shown to substantially increase urinary 1-M-histidine excretion [114]. The standardized dinner resulted in similar 1-M-histidine concentrations in urine and plasma at the beginning of both blocks. This finding clearly established that 1-M-histidine levels in plasma and in urine can serve as a marker of meat intake while 3-M-histidine levels depend also on body muscle mass [18, 109].

Overall, we can conclude, that the over-night fasting metabolome in plasma and urine is quite robust in healthy volunteers. The amino acid metabolome is a metabotype-specific entity under anabolic conditions, but not in the extended fasting state. In addition, the specific study design identified 1-M-histidine in urine and plasma as a surrogate marker for meat intake.

3.2. BCAA and α-aminobutyrate as potential markers for the metabolic condition

Over the entire study, the time-course profiles of the 3 plasma BCAA were of similar type. This resulted in strong cross-correlations and also grouping by a HCA. Plasma phenylalanine and α-aminobutyrate levels were strongly associated with BCAA profiles. Thus, independent of the metabolic state, the dynamics as well as the concentrations of these amino acids seem to be tightly coupled. The OGTT and the extended fasting resemble opposed metabolic conditions and therefore this subset of metabolites can serve as surrogates that mark with characteristic changes an anabolic or a catabolic condition. In the anabolic state with elevated insulin levels, these amino acids decline in plasma whereas during extended fasting they increase progressively. After glucose administration, insulin concentrations increase and this shifts the metabolism from catabolism to anabolism. The plasma BCAA concentrations but also levels of other amino acids decrease substantially probably by insulin action on membrane amino acid transporters in tissues such as muscle

and liver [24, 25, 115]. BCAA and phenylalanine share specific transporters, for instance the L-type amino acid transporter 1 and 2 (LAT1, LAT2) [116]. Based on a bioinformatical approach, plasma changes of BCAA and aromatic amino acids in response to an OGTT were linked to LAT 2 transport activity [61]. Healthy volunteers ingested a mixture of essential amino acids and thereafter muscle biopsies were collected. Drummond *et al.* showed next to increased plasma insulin levels, that leucine concentrations and transcript levels of LAT1, the sodium-coupled neutral amino acid transporter 2 and the proton-coupled amino acid transporter 1 are increased in the muscle of these volunteers [117]. These processes may also cause the changes in plasma amino acids during the OGTT observed in the HuMet-study. Even 240 min after glucose administration – when glucose and insulin levels long had declined to baseline levels – amino acid levels (BCAA, aromatic amino acids and others) were still below baseline concentrations. This became also visible in the PCA score plot as exemplified for the trajectories of two individuals. Aoki demonstrated similar long-lasting effects with increased valine and isoleucine influx rates into forearm muscle up to 4 hours after challenge [50]. Furthermore, the observed metabotype specific grouping for the anabolic challenges suggested a volunteer specific transport regulation, which may indicate differences in amino acid transport activation by insulin and therefore in insulin sensitivity.

The OGTT is one of the most simplistic challenge but is also highly standardized [74]. Although one could argue that it does not resemble a meal, it allows to assess the systemic insulin effects on the endogenous metabolome without interference of dietary components (glucose, amino acids, lipids) that enter systemic circulation after meal ingestion. As demonstrated, mice submitted to an OGTT displayed similar changes in plasma as humans but here the additional assessed amino acids in skeletal muscle and liver revealed only slight changes. However, those were unexpected by reductions rather than elevations. BCAA species in plasma of mice showed reductions between 60 to 80 µmol/l. Male adult C57BL/6 mice as gavaged in the experiment have a body weight of about 25 gram, while lean body mass accounts for approximately 18 g [118]. Based on a 25 g mouse with a blood volume of 2 ml (about 1 ml of plasma), an oral glucose load leads to an apparent removal of 60 to 80 nmol of the BCAA by the action of insulin. This is mainly by the uptake into muscle, but amino acid transporters in adipocytes may also be affected by insulin [115]. The amount of BCAA taken up into tissues is most likely too small to detect corresponding

alteration in the tissues. Insulin action on tissues also reduces protein breakdown [22] and promotes in the presence of sufficient amino acids an upregulation of protein synthesis [23]. The slightly reduced amino acid concentrations in skeletal muscle and liver 30 min after glucose administration may therefore derive from a stronger effect of insulin action on suppression of protein degradation than on increased uptake from the extracellular space.

While in response to an OGTT the majority of amino acids declined in the HuMet-volunteers, the extended fasting challenge induced a more diverse amino acid response pattern. The fasting period of 36 hours leads to elevations of plasma BCAA, α-aminobutyrate and phenylalanine levels and caused therefore opposing changes when compared to the OGTT. These trajectories marking the two opposed metabolic conditions were also observed in mice. Similar fasting induced elevations of α-aminobutyrate and BCAA were previously reported for humans and rodents [78, 82, 119-122]. We assume that α-aminobutyrate underlies similar transport phenomena and regulation principles as BCAA and phenylalanine; yet corresponding studies for example on α-aminobutyrate transport are missing. The endogenously produced amino acid α-aminobutyrate is a product of methionine and threonine metabolism and formed via the intermediate α-ketobutyrate [66]. Its breakdown involves a transamination step [101], however it is currently not known which transaminase mediates this process. Nevertheless, it is interesting to note that in *Arabidopsis thaliana* α-aminobutyrate serves as a substrate of the mitochondrial branched-chain amino acid aminotransferase (BCAT) [123]. Moreover, α-ketobutyrate was shown to be metabolized in bovine kidney by branched-chain α-keto acid dehydrogenase complex (BCKD) [124], the rate limiting enzyme in the degradation of the BCAA-derived keto acids after transamination. Although currently hypothetical, the literature and the coherent time-profiles of α-aminobutyrate and BCAA found throughout the HuMet-study suggest that common pathways with identical enzymes could be involved in the metabolism of α-aminobutyrate and BCAA.

Mice models could in future be useful to reveal the mechanisms underlying these phenomena and we therefore induced a catabolic state in mice by a fasting period of 12 hours (over-night) and 24 hours respectively. However, both fasting periods resulted in similar metabolic alterations, probably because mice are nocturnal animals. The plasma amino acid changes induced by fasting were comparable

between humans and mice. CN analysis revealed that the plasma pattern was linked to changes observed in skeletal muscle tissue, as previously reported by Adibi [119]. Both compartments displayed for instance highly associated and coherent increases in BCAA and α-aminobutyrate. The α-aminobutyrate-precursor α-ketobutyrate is a pyruvate homolog and can further be metabolized to 2-OH-butyrate. Alternatively α-ketobutyrate can be decarboxylated to propionyl-CoA via α-ketobutyrate-dehydrogenase, an enzyme with high activity in liver tissue [125]. NMR analysis of mouse fasting plasma revealed, that 2-OH-butyrate levels markedly increased similar to the levels of ketone bodies such as 3-OH-butyrate and acetoacetate. Ketone bodies are known to inhibit α-ketobutyrate-dehydrogenase activity [125], which may shift the system towards the formation of α-aminobutyrate and 2-OH-butyrate. Previously, it was reported that 2-OH-butyrate levels are highly negatively correlated to plasma threonine levels and are positively associated with its product α-aminobutyrate and this finding suggested that threonine is the main precursor of these products. [82]. Although 2-OH-butyrate levels were not associated to threonine concentrations in plasma of fasted mice, there were associations found with α-aminobutyrate levels measured in skeletal muscle, liver and plasma. Ketone bodies and fatty acids have been shown to reduce the catabolism of threonine via threonine-dehydrogenase pathway [126]. This may result in higher threonine degradation via the second threonine metabolizing enzyme, the threonine-dehydratase leading ultimately to the formation of α-ketobutyrate. A dietary excess of threonine resulted in elevated plasma threonine and α-aminobutyrate concentrations in adults and further threonine-dehydrogenase seems to play a minor role in threonine oxidation in adults compared to the threonine dehydratase pathway [127]. The observed increases of α-aminobutyrate observed in the current work therefore most likely result from changes in threonine catabolism and in α-ketobutyrate-dehydrogenase activity. The proposed mechanisms of alterations and the link to the BCAA metabolism lead to this distinct plasma marker metabolite of anabolic or catabolic conditions.

In mouse liver, next to α-aminobutyrate β-aminoisobutyrate levels increased during fasting. β-aminoisobutyrate is a thymine catabolite and is associated with increased hepatic fatty acid oxidation and a reduction of fat mass in mice [128]. Urinary excretion of β-aminoisobutyrate increased in volunteers of the HuMet-study by fasting together with a progressive elevation of plasma NEFA suggesting a high rate of lipolysis and fatty acid oxidation [98]. This higher provision of NEFA derived from

lipolysis to muscle energy metabolism during fasting is mirrored by increased levels of plasma long-chain acylcarnitine species in humans and mice. Interestingly, plasma concentrations of free carnitine (C0) and acetyl-carnitine (C2) in volunteers and mice were highly negatively or positively associated with concentration changes of BCAA and α-aminobutyrate in anabolic or catabolic conditions. Oxidation of fatty acids in mitochondria requires activation of fatty acids by the long-chain acyl-CoA synthetase at the mitochondrial outer membrane. The carnitine system involves the carnitine palmitoyltransferase 1, the carnitine:acylcarnitine translocase (CACT) and the carnitine palmitoyltransferse 2 (CPT2) and shuttles activated fatty acids (acyl-CoAs) from the cytosol into the mitochondrial matrix as acylcarnitines. At the inner mitochondrial membrane a re-conversion to acyl-CoAs by CPT2 takes place and acyl-CoAs are submitted to β-oxidation [129]. The oxidation of acyl-CoAs leads to formation of acetyl-CoA that finally enters the TCA cycle. When fatty acid import into mitochondria exceeds β-oxidation capacity, a “spill-over” of the system becomes visible by appearance of C2 in the extracellular domain. An excess of acetyl-CoA is converted to C2 via carnitine acetyltransferase which can exchange with carnitine via the mitochondrial CACT shuttle and C2 appear in circulation [130]. Consequently, in conditions of enhanced β-oxidation there is an increased demand for C0. In the fasting state, synthesis of C0 in liver and kidney as well as C0 uptake was enhanced in pigs [131] and Luci *et al.* showed similarly in rats an upregulation of the carnitine transporter [132]. The initial step of carnitine biosynthesis occurs in muscle as well [133]. Lysine is the precursor for carnitine and reduced lysine levels found in skeletal muscle of fasted mice supported an enhanced biosynthesis. Therefore, the opposed concentration changes of plasma C2 and plasma C0 found in mice and humans during fasting reflect the provision of C0 to β-oxidation in the catabolic state and the release of C2 as a “spill-over” phenomenon. Studies on extended physical exercises in humans also reported such an anti-correlation of C0 and C2 levels [107].

Next to the enhanced rate of β-oxidation in the catabolic condition, an upregulation of hepatic gluconeogenesis was also reflected in the amino acid profiles of fasted mice as liver amino acid concentrations and gluconeogenic amino acids like alanine, serine and glycine measured in skeletal muscle were reduced. Furthermore, the diminished plasma concentrations of pyruvate and lactate suggested *de novo* glucose production. Alanine in the center of the CN illustrates its important role in providing glucose to the periphery within the glucose-alanine cycle during catabolism.

So far, we can conclude that the anabolic hormone insulin influences the plasma amino acid response after an OGTT. Insulin activates amino acid transporters that mediate the amino acid uptake into tissues and result in a measurable decline of plasma concentrations. For the fasting challenge, our metabolite data pictured several axes of metabolic adaptations during fasting: an upregulation of proteolysis, lipolysis, ketoneogenesis and gluconeogenesis, as BCAA, α-aminobutyrate, hepatic fatty acids, acylcarnitines and ketone bodies increased and gluconeogenic compounds decreased. Most pronounced changes were found for BCAA and α-aminobutyrate. These metabolites displayed an opposed trajectory in both metabolic states and seemed to underlie similar regulation mechanisms. Thus, both conditions cause coherent and strongly correlated changes in these amino acids but also in acylcarnitines that subsequently mark the given metabolic state. Although, the underlying mechanisms that could explain these linked changes are largely elusive, there are plausible biochemically links in metabolic pathways that need to be verified experimentally.

3.3. Can changes in the healthy metabolome help to understand metabolic alterations in the disease state?

In clinical practice, diabetes is diagnosed by the measurement of plasma glucose concentrations after an over-night fast or 2 hours after an OGTT [74]. As the incidence of diabetes increases epidemically, there is a need for additional biomarkers. Herder *et al.* reviewed the potential of novel diabetes biomarkers (C-reactive peptide, leptin, triglycerides *etc*.) and showed that these new markers improved the clinical risk score of classical markers (glucose, impaired glucose tolerance, glycated hemoglobin) only slightly [134]. One reason why new markers often failed to improve risk scores is the fact that for most of them no clear “cut-off” values for health and disease states exist. It was therefore argued that “new biomarkers” should be independent from established markers [134]. Established biomarkers like elevated blood glucose diagnose more or less an existing disease state and prognostic biomarkers are needed that predict an onset of a disease before it becomes manifest.

Already in the late 1960s, studies showed that plasma BCAA concentrations are increased in obese subjects [56, 100]. Similar changes were shown for type 1 and

type 2 diabetic patients [57-59]. In recent years, several targeted and untargeted metabolomics approaches have also identified BCAA as the most discriminating metabolites for the diabetic state and assigned them as potential biomarkers. Table 10 presents an overview of the corresponding studies.

Amino acids meet the criteria to be independent to the classical diabetes biomarkers like glucose and glycated hemoglobin levels. Wang and coworkers showed in 2011 that early changes in BCAA together with phenylalanine and tyrosine levels are able to predict future diabetes in two cohorts [12]. In contrast, a recent prospective population-based study identified glycine together with C2 and lysophosphatidylcholine 18:2 as predictive markers within the Cooperative Health Research in the Region of Augsburg cohort. Although significant BCAA levels were detected as well, the authors failed to confirm the predictive power of BCAA [135] as shown by Wang *et al.* [12]. Most likely different study designs lead to this discrepancy. Other metabolomics studies revealed metabolites such as ketone bodies, 2-OH-butyrate, NEFA and acylcarnitines as additional discriminative markers for obesity, insulin resistance and T2DM (Table 10). It is of note, that an insulin deficiency (type 1 diabetes) as well as impaired insulin sensitivity as in obesity, insulin resistance and type 2 diabetes resulted in similar increases in BCAA concentrations. As the metabolic outcome of obesity and type 2 diabetes is comparable (Table 10), only the term diabetes will be used in the upcoming section and no differentiation will be made between the actual disease conditions.

Most interestingly, there is an impressive overlap of metabolites that change in diabetic patients and metabolites defining the fasting state in healthy humans. This includes changes in BCAA, NEFA, acylcarnitines, ketone bodies and 2-OH-butyrate. As shown by Krug *et al*. insulin concentrations declined during the fasting trial in the HuMet-study [98] and fasting induced a transient peripheral insulin resistance in healthy volunteers [136]. Elevation of BCAA in the fasted state as shown in the present work was already described in 1969 [78]. As depicted in Figure 5, energy requirements during fasting are matched through increased lipolysis, β-oxidation, proteolysis, ketoneogenesis and gluconeogenesis. In the healthy human, insulin suppresses all these metabolic pathways and therefore reverses the changes in marker metabolites into the opposite direction. This however is impaired in states of insulin resistance and diabetes. In the diabetic state, the organism “remains in the fasting condition” despite elevated insulin levels but due to the impaired insulin

Table 10: Overview of selected studies investigating metabolome changes in obesity, insulin resistance and type 2 diabetes
In all studies listed here, collection of plasma/serum samples took place after an over-night fast. Urine samples that were investigated by [13] were spot urine samples. # metabolites that are predictive for future diabetes.

Study	Year	Subjects	Bio fluid	(Selected) Discriminating metabolites
Adibi *[56]*	1968	obese female	plasma	valine, leucine, isoleucine
Felig *et al.* [100]	1969	obese subjects	plasma	valine, leucine, isoleucine, phenylalanine, tyrosine
Wahren et al. [58]	1972	type 1 diabetics	plasma	valine, leucine, isoleucine
Vannini *et al.* [57]	1982	type 1 and type 2 diabetics (poorly controlled)	plasma	valine, leucine, isoleucine
Marchesini et al. [59]	1991	male type 2 diabetics	plasma	valine, leucine, isoleucine
Manders *et al. [137]*	2006	male type 2 diabetics	plasma	valine, leucine, isoleucine
Newgard *et al.* [15]	2009	obese subjects	serum	valine, leucine/isoleucine, phenylalanine, tyrosine, propionylcarnitine (C3), isovalerylcarnitine (C5)
Fiehn *et al.[8]*	2010	female type 2 diabetics	plasma	valine, leucine, long chain fatty acids
Gall *et al.[60]*	2010	nondiabetic subjects (normal and impaired glucose tolerant)	plasma	2-OH-butyrate
Menge *et al. [138]*	2010	impaired glucose tolerant subjects and type 2 diabetics	plasma	valine, leucine
Mihalik *et al. [65]*	2010	female type 2 diabetics	plasma	acylcarnitine species (total sum, acetylcarnitine (C2), propionylcarnitine (C3), etc.)
Suhre *et al. [11]*	2010	male type 2 diabetics	plasma	valine, leucine, isoleucine, 3-OH-butyrate
Zhao *et al.* [13]	2010	impaired glucose tolerant subjects	plasma/urine	fatty acids, lysophospholipids, acylcarnitines
Wang *et al.* [12]	2011	normoglycemic subjects	plasma	# valine, leucine, isoleucine, phenylalanine, tyrosine
Wang-Sattler *et al.* [135]	2012	impaired glucose tolerant subjects	serum	# glycine, lysophosphatidylcholine (18:2), acetylcarnitine (C2)

sensitivity of peripheral tissues and liver. What however makes the BCAA the “most sensitive” metabolites with predictive quality that 12 years before a diabetes becomes manifest change [12], remains to be defined. Yet, the similar increases observed under fasting conditions in healthy humans and mice may shed some light on possible underlying biochemical processes.

The key enzymes involved in metabolism of BCAA such as BCAT and BCKD are expressed in tissues such as liver, skeletal muscle and adipose tissue [139, 140]. Fukuhara *et al*. proposed that during fasting BCAA metabolism in muscle produces the corresponding keto acids that are subsequently transported to liver. Here a reconversion to BCAA takes place and finally BCAA are released back into circulation leading to higher levels in plasma [141]. The reconversion of BCKA into BCAA in hepatocytes would require a substantial BCAT activity, but this seems to be rather low in hepatocytes [140]. Moreover, the reduced hepatic BCAA concentrations in fasted mice rather suggest an enhanced catabolism or flux than a reconversion of the BCKA to BCAA. In a perfused rat liver model, hepatic leucine was released during starvation [120]. Rate-limiting for BCAA degradation is the irreversible reaction by BCKD. The dehydrogenase complex is inactivated by the action of the branched-chain α-keto acid dehydrogenase kinase (BCKDK) via phosphorylation [54]. The loss of BCKDK results in a severe reduction of plasma BCAA concentrations and growth retardation [142]. Shimomura *et al*. reviewed the regulation of BCKD via BCKDK [54]. Important mechanisms to be considered are: i) there is an increased BCKD activity during starvation due to decreased kinase activity in liver and ii) increased fatty acids levels may mediate a downregulation of BCKDK via activation of peroxisome proliferator-activated receptor α (PPAR-α). Indeed, higher concentrations of hepatic fatty acids were found in fasted mice. Amongst the fatty acids with elevated levels was also 20:5(n-3) which is known to activate PPAR-α [143]. iii) The link to the diabetic state is provided by the findings that BCKDK activity was downregulated in liver and skeletal muscle what consequently results in increased BCKD activity. This however should increase the oxidation of BCAA and not increase their plasma levels.

Besides elevated BCAA, high concentrations of acylcarnitines were also identified as marker metabolites for the diabetic state [15, 65, 71]. The acylcarnitines C3 (propionyl-carnitine) and C5 (valeryl-carnitine) are breakdown products of BCAA. Newgard recently hypothesized, that a “spill-over” of BCAA catabolism results in enhanced C3 and C5 formation and elevated BCAA levels. Furthermore, succinyl-

CoA and acetyl-CoA are products of BCAA breakdown that can enter the TCA cycle. But these metabolites might also be potential inhibitors of β-oxidation [55]. In both, the HuMet-study as well as in mice, we found no correlation between plasma BCAA concentrations and plasma C3 and C5 levels in the fasted state arguing against the Newgard concept [55]. Nevertheless, the anti-correlation of C0 and C2 as well as the increase of long-chain acylcarnitines suggest increased β-oxidation in fasting state, which may inhibit BCAA breakdown by the increased levels of free fatty acids, as proposed recently [64] and ketone bodies. It thus would be interesting to profile acylcarnitines concentrations especially in skeletal muscle of fasted mice.

Adams argues that a NEFA-rich environment inhibits mitochondrial BCKD activity [64]. Enhanced β-oxidation results in an increase of the NADH/NAD^+ ratio and accumulation of acetyl-CoA that inhibits BCKD, similar to the inhibition of the pyruvate dehydrogenase complex (PDH) [64]. NEFA concentrations in plasma increased in the HuMet-fasting trial [98] and higher levels of long-chain acylcarnitines in fasted mice and humans also suggests enhanced rates of β-oxidation. Since BCKD may also metabolize α-aminobutyrate and α-ketobutyrate, the inhibition of this enzyme by acetyl-CoA could also promote the conversion of α-ketobutyrate either into α-aminobutyrate or into 2-OH-butyrate. Like in fasted volunteers shown here and in a previous study [82], elevated α-aminobutyrate concentrations are found in insulin-deficient mice (Pieter Giesbertz, Molecular Nutrition Unit, Technische Universität München, unpublished data). Furthermore, our fasted mice displayed high 2-OH-butyrate concentrations, a metabolite classified as a discriminator for the diabetic state by random forest analysis [60].

Taken together, the impairment of BCAA handling as described in diabetes and in the fasting state in healthy mammals, appears to have the same origin. High levels of fatty acids and acetyl-CoA under these conditions probably inhibit BCKD activity. This also may lead to an alternative rout of utilization of α-ketobutyrate in both states that forms the basis of the inherent close association of these metabolites under all conditions. In the fasting and diabetic state the breakdown of essential BCAA therefore seems to be suppressed as also shown previously by a reduced oxidation of leucine in adipose tissue [144]. Furthermore, when BCKD is inhibited, products such as C3 and C5 would not increase as proposed by Newgard [55]. Although we did not found any association between BCAA, C3 and C5 during fasting, whether this is different in diabetes needs to be established.

In search of proper mouse models that may lead to the characteristic changes in the plasma metabolome as in insulin-resistant or diabetic humans, we employed a DIO model using C57BL/6 mice. Although animals from all feeding trials turned obese and displayed elevated fasting glucose levels, the plasma metabolite profiles did not resemble what has been reported in humans, particularly when BCAA levels are considered. Unaltered BCAA in wildtype DIO mice were reported by others as well [62, 145]. Nevertheless, elevated concentrations of BCAA were found in leptin-deficient ob/ob mice [139] and in leptin receptor-defective zucker diabetic fatty (ZDF) rats [146]. It is of course tempting to speculate that the lack of leptin respectively proper leptin signaling may be involved in the cause of elevated plasma BCAA concentrations in humans. Leptin administration is able to reduce plasma NEFA concentrations in non-obese diabetic mice and can normalize hepatic BCAA concentrations similar to insulin therapy [147]. Mice with disturbed leptin signaling may miss therefore the NEFA-lowering action of leptin. Moreover, leptin levels are increased in HF C57BL/6 mice and these mice do not display changes in plasma triglyceride concentrations [62, 145], but have reduced total NEFA [145]. To which extent leptin is involved in the regulation of NEFA concentrations and consequently in the elevation of BCAA in humans has to be studied. Obese subjects have increased plasma NEFA and BCAA concentrations accompanied by elevated leptin levels [15] and most interestingly leptin levels drop drastically in obese and lean subjects during early starvation [148].

Thus, findings on the links of diabetes to BCAA metabolism are conflicting. The model developed by Adams based on results obtained by metabolomics approaches [64] and contrasts with the findings reported by Shimomura *et al*. reporting an activation of BCKD during fasting and in diabetes. Yet, those data are mainly from rats [54]. Enzyme activities of BCAA-metabolism differ significantly between species and tissues [140]. She *et al.* investigated in different obesity models, the expression and activity of BCAT and BCKD. They showed, that elevated BCAA concentrations were associated with reduced enzyme activities in liver and fat tissue [139]. In adipose tissue of mice with a specific overexpression of the glucose transporter 4 a reduced BCAA oxidative capacity was reported and it was shown that this substantially influenced circulating BCAA concentrations [149]. Therefore, future experiments should address the contribution of different tissues to alterations in circulating BCAA concentrations and especially the role of adipose tissue has to be

re-considered. Experiments using isolated tissues [117, 139], for instance with human biopsies or with isolated tissues from animal models after defined periods of fasting would be an appropriate approach to determine alterations of enzyme activities of BCAT and BCKD as well as metabolite concentrations. Future analysis should also include the quantification of the BCAA-derived α-ketoacids and NEFA in plasma and tissues.

Our data obtained in fasting humans and mice supports the model of Adams [64]. Central to that is that a NEFA-rich environment promotes metabolic alterations leading to the BCAA elevations observed and their prognostic value. Metabolic "fingerprints" of diabetes and the fasting condition in healthy humans are remarkably similar or even identical and this may have its origin in changes derived from impaired insulin signaling. However, it is of note to mention that the impairments of insulin signaling may underlie different mechanisms. Whereas insulin resistance is associated with reduced insulin-mediated Akt (known as protein kinase B) phosphorylation status as shown for example in db/db mice [150] a fasting period over 72 hours causes also insulin resistance but is associated with reduced phosphorylation of AS160, a substrate of Akt, but not of Akt itself [151]. Future studies including human trials with obese, insulin resistant and/or prediabetic volunteers as well as genetic animal models (db/db mice, ob/ob mice, ZDF rats) are needed to systematically assess at which state the changes in amino acid metabolism take place and why the amino acids are superior markers of diabetes.

3.4. Are citrulline/arginine metabolism also linked to diabetes?

Besides BCAA metabolism, there is evidence that citrulline/arginine metabolism is impaired in diabetic conditions. We found early elevations in plasma citrulline concentrations in the mouse DIO models associated with hyperglycemia in the HF-feeding trials. Furthermore, the urea cycle intermediate ornithine was increased. Elevated citrulline concentrations were described previously in different mouse models for obesity and diabetes [139, 152, 153], whereas citrulline was reduced in ZDF rats [146]. In humans, reports on plasma citrulline concentrations in the diabetic state reveal controversial findings [14, 15, 137], but in 2011 Verdam *et al.* showed that increased citrulline concentrations were strongly associated with elevated levels of glycated hemoglobin [154].

Citrulline is linked to arginine metabolism and is thus important for the maintenance of systemic arginine concentrations [39]. In plasma as well as in skeletal muscle arginine bioavailability expressed as arginine/(ornithine + citrulline) was diminished in all DIO animals. This is in agreement to previous reports in mice and diabetic patients [104, 155] and lower arginine concentrations were reported in diabetes [137, 138]. Supplementation trials with arginine/citrulline increased systemic concentrations of both urea cycle intermediates [156, 157] and that led to improved glucose homeostasis in insulin-deficient rats [157], while glucose concentrations decreased in obese ZDF rats [156]. Arginine can either be metabolized via arginase or via the NO-synthases. Increased systemic arginase activity was reported for type-2 diabetics and for DIO mice [104, 155]. However, it is not likely that high arginase activity results in the observed increases in citrulline levels. Although, the K_M is in a low millimolar range for arginase and in a low micromolar range for NO-synthase [158], the activity of arginase is 1000 times higher than NO-synthase activity at physiological pH [158]. An upregulation of arginase activity in the diabetic state may therefore reduce substrate availability for NO-synthases and would subsequently result in a lower conversion of arginine to NO and its byproduct citrulline.

Citrulline is recycled to arginine in endothelial cells [159]. This pathway involves the enzymes arginino succinate-synthetase and arginino succinate-lyase. *In vitro*, an insulin-mediated upregulation of transcripts for arginino succinate-synthetase and NO-synthase in endothelial cells in culture was reported and endothelial cells isolated from insulin-deficient rats conversely displayed a downregulation of these enzymes [160]. Assuming that an impaired insulin action in obese mice may induce similar regulatory mechanisms than insulin deficiency, a suppression of citrulline to arginine conversion may cause the elevated systemic citrulline concentrations.

Citrulline possesses a unique inter-organ metabolism comprising small intestine, kidney and liver. In particular, enzymes of the urea cycle take part in biochemical processes related to the inter-organ exchange (see Figure 2). Plasma citrulline concentrations associated with intestinal dysfunction [161] and upregulated transcript levels of intestinal arginase II and carbamoyl-phosphate synthetase 1 in DIO mice [103] also suggested a higher intestinal production rate of citrulline. However, this could not be demonstrated in DIO mice when the intestine in vitro was supplied luminally with stable-isotope labeled arginine. Except for carnosine, renal amino acid concentrations remained also unchanged, although renal insufficiency and

dysfunction were linked to elevated plasma citrulline concentrations [162, 163]. The increase in systemic citrulline levels could therefore not be linked to the citrulline-metabolizing tissues such as small intestine and kidney. Controversial findings exist if hepatic citrulline metabolism is linked to the systemic metabolism [47, 164, 165]. The question is, of whether hepatic release or uptake of citrulline contributes to the observed plasma citrulline increases in DIO mice. Hepatic amino acid analysis revealed reduced ornithine concentrations, but no change in citrulline levels in DIO mice and similar to others [49] arginine was below detection. Since DIO causes usually enhanced hepatic glucose production [105, 106] from glucogenic precursors such as the amino acids increased urea cycle activity for detoxification of ammonia is required. Early hepatic insulin resistance is characterized by a high rate of glucose synthesis and already short term HF interventions in C57BL/6 mice cause elevations in hepatic aspartate and other metabolites related to the urea cycle [166]. In our DIO mice hepatic ornithine concentrations were significantly associated with glucogenic amino acids levels (when alanine and glutamine were omitted) and a negative association between hepatic ornithine levels and plasma citrulline concentrations was obtained. This could suggest that there is an exchange between these compartments. Enzymes involved in the urea cycle are located partly in the mitochondria and in the cytoplasm. Indiveri and coworkers characterized an ornithine/(citrulline + H^+)-exchanger that ensures the mitochondrial efflux of citrulline. The influx of ornithine into mitochondria is essential for the function of the urea cycle [167]. Although no transporter involved in the proposed hepatic uptake/release of citrulline could be identified to far, it may be speculated that a transporter with similar properties as the identified ornithine/(citrulline + H^+)-exchanger in the plasma membrane could cause the plasma citrulline elevations.

In DIO animals, plasma hydroxyproline concentrations fell already after 6 weeks of dietary intervention and diminished concentrations were found in liver as well. Previously, Wijekoon *et al*. reported in ZDF rats reduced hydroxyproline levels [146]. In type 2 diabetics, urinary hydroxyproline excretion is increased [168, 169] and may explain the observed reductions in plasma hydroxyproline concentrations. Hydroxyproline can be metabolized into pyruvate and serves thereby as a gluconeogenic substrate. As endogenous glucose production is increased in fasting [22, 76], the decreased levels of hydroxyproline in plasma found in the HuMet-study

as well as in fasted mice may indicate its role as a precursor for glucose production in liver.

To conclude, elevated citrulline concentrations and diminished arginine bioavailability indicate that the citrulline/arginine metabolism is systemically impaired in DIO mice. This may have its origin in a higher urea cycle activity in DIO mice and a unique exchange of hepatic ornithine with systemic citrulline. Further experiments should address if indeed an ornithine/citrulline-exchange between hepatic tissue and plasma takes place and human studies are needed to assess whether citrulline metabolism is involved in obesity related diseases like diabetes.

3.5. Conclusion and future perspectives

Based on our findings we can conclude that the amino acid pool within the metabolome is tightly regulated. Both, anabolic and catabolic conditions change systemic amino acid patterns in a characteristic manner with a close interrelationship in particular the BCAA, phenylalanine and metabolites derived from threonine metabolism. The response pattern in the anabolic condition seems particularly under strong control by insulin. Almost all metabolites that change under extended fasting conditions in healthy humans and mice belong to the group of metabolites that are considered as “biomarkers” of obesity and diabetes. This observation may have a common origin as proposed by Adams [64]. According to this model, a NEFA-rich environment causes an inhibition of BCDK, a key enzyme controlling BCAA metabolism. Its inhibition could be responsible for the elevation in BCAA levels, as well as the increased formation of α-aminobutyrate and 2-OH-butyrate. Although C57BL/6 DIO mice show some features such as systemic and hepatic changes in citrulline/arginine metabolism as occasionally observed in human diabetic states, they seem not the proper model to assess the metabolic impairments that lead to the characteristic changes in the metabolome in human diabetes.

As revealed by the metabolite profiling approaches in the HuMet-study, metabotype-specific concentration changes – marking individual’s – can be derived by challenge tests. Although the volunteer group was highly homogenous by age, gender, BMI and other measures and showed only minor variations in basal (over-night fasting) levels of metabolites, any challenge caused major deviations in metabolite profiles amongst the individuals in the time-trajectories. This demonstrates that the metabolic adaptation to catabolic or anabolic conditions creates distinct individual variations supporting the concept of “metabolic flexibility” of individuals, which is markedly different. Challenge tests as described here may therefore be particularly helpful in comprehensive phenotyping approaches for example to link genetic heterogeneity to phenotypic responses or in diagnostic approaches to reveal new biomarkers.

4. Material & Methods

4.1. Chemicals

All chemicals were purchased from Roth (Karlsruhe, Germany), Sigma (Taufkirchen, Germany) or Merck (Darmstadt, Germany) unless not stated otherwise.

4.2. Mouse studies

4.2.1. High-fat feeding trials

Each HF intervention trial was performed using male C57BL/6 mice and the intervention period lasted twelve weeks. All animals had access to food and tap water *ad libitum* and maintained in a 12 h light/dark cycle. Blood was collected under anesthesia into EDTA- or lithium-heparin coated tubes (both Sarstedt, Nümbrecht, Germany) by puncture of the retro-orbital sinus before mice were sacrificed via cervical dislocation. After incubation on ice for 30 minutes, blood was centrifuged (3000 x g) for 10 min at 4°C. The obtained plasma and tissue samples were immediately snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Study 1:

Embedded in the NuGO PPS3, this HF feeding trial, which was described previously [102, 170] was performed at Wageningen University (Wageningen, the Netherlands). The Local Committee for Care and Use of Laboratory Animals at Wageningen University approved the study (study number 2007118.b). Male C57BL/6J mice were purchased from Charles River (Maastricht, The Netherlands) at the age of three weeks and housed individually. At the age of 12 weeks, mice were fed the control diet (10 E% of fat) for four weeks as a run-in period. Afterwards, animals were randomized into two groups (n=12) and fed either a HF diet (45 E% of fat) or were continued to feed the control diet. Both diets were based upon Research Diets formulas (www.researchdiets.com; D12450B / D12451) and palm oil was the major fat source (Table 11). Throughout the feeding trial body weight and food intake were monitored weekly and EDTA-plasma was collected every second week. At the end of the intervention period, mice were fasted for five hours, anaesthetized with a mixture

of isoflurane (1.5%), nitrous oxide (70%), and oxygen (30%) and blood and liver samples were collected for amino acid analysis.

Table 11: Nutrient composition of experimental diet (study 1)
Nutrient composition is expressed as (g/kg), unless not stated otherwise.

	10 E% (Control)	45 E% (High-fat)
Crude protein	200	200
Crude fat	45	202.5
Soybean oil	25	25
Palm oil	20	177.5
Crude fiber	50	50
Starch	427.2	72.8
Sugar	172.8	172.8
Maltodextrin	100	100
Gross energy (MJ/kg)	15.9	19.7
% carbohydrate	69	35
% protein	20	20
% fat	10	45

Study 2:

The HF intervention was performed as described previously [171], conducted according to the German guidelines of animal care and approved by the state ethics committee (reference number 209.1/211-2531-41/03). C57BL/6N mice were purchased from Charles River Laboratories (Sulzfeld, Germany) at the age of 8 weeks, housed individually and fed a standard laboratory chow (V1534-0, Ssniff GmbH; Soest, Germany) (Table 12) for two weeks. At the age of 10 weeks, mice were randomized into two groups (n=9): one control group (11 E% of fat) and one HF group (60 E% of fat). All diets were purchased from Ssniff GmbH (E1534 for standard chow, E15000-04 for control diet, E15741-34 for HF diet; Soest, Germany) (Table 12). After 12 weeks of dietary intervention, non-fasted mice were anaesthetized with isoflurane (Baxter, Unterschleißheim, Germany), and sacrificed after plasma was collected into lithium heparin coated tubes (Sarstedt, Nümbrecht, Germany). The small intestine was immediately taken out and used for gut sacs experiment as described below. In addition to plasma, liver, skeletal muscle, kidney and scraped mucosa were sampled for amino acid analysis.

Study 3:

C57BL/6J mice were housed in pairs or in threesomes. At the age of 8 weeks, mice received the control diet (12 E% of fat) for four weeks. At the age of 12 weeks, mice were randomized into two groups. During the intervention period, mice received either a control (12 E% of fat, n=6) or a HF (48 E% of fat, n=5) diet. Both diets were purchased from Ssniff GmbH (S5745-E702 for control diet, S5745-E712 for high-fat diet; Soest, Germany) (Table 12). After 12 weeks, non-fasted mice were anaesthetized with isoflurane (Baxter, Unterschleißheim, Germany). Plasma was collected into lithium heparin coated tubes (Sarstedt, Nümbrecht, Germany) before mice were sacrificed. Plasma was used for metabolite detection via GC-MS.

Table 12: Nutrient compostition of experiment diets of the high-fat studies 2 and 3 and the metabolic challenges fasting and OGTT in mice
Nutrient composition is expressed as (g/kg), unless not stated otherwise.

	Study 2		Study 3		Chow
	C	HF	C	HF	
Crude protein	208	241	208	208	190
Crude fat	42	340	51	251	33
Beef tallow	-	310	-	-	-
Soybean oil	40	30	50	50	-
Palm oil	-	-	-	200	-
Crude fiber	50	60	50	50	49
Starch	468	22	478	278	366
Sugar	108	224	50	50	47
Maltodextrin	-	-	56	56	
Gross energy (MJ/kg)	18	25.2	16.9	21.9	16.3
Metabolizable energy (MJ/kg)	15	21.4	15.5	19.7	12.8
% carbohydrate	66	21	65	34	58
% protein	23	19	23	18	33
% fat	11	60	12	48	9

4.2.2. Metabolic challenges in mice

C57BL/6 mice were maintained at 24°C in a 12:12 h light/dark cycle and fed a normal chow diet (V1534-0, Ssniff GmbH, Soest, Germany) (Table 12). The study was conducted according to the German guidelines of animal care and approved by the state ethics committee (reference number 55.21.54-2532-45.11). Blood glucose was measured from the tail using an Accu-Check blood glucose meter (Roche Diagnostics, Mannheim, Germany). Afterwards, mice were anesthetized with isoflurane (Baxter GmbH, Oberschleißheim, Germany). For amino acid analysis

blood was collected from the retro-orbital sinus. Blood samples were centrifuged (3,000 x g, 10 min, 4°C) and plasma was directly aliquoted on ice. After killing the mice by cervical dislocation liver and skeletal muscle samples were collected and snap frozen in liquid nitrogen. All samples of the fasting and the OGTT were stored at -80°C until analysis.

Fasting challenge

Before fasting 24 animals between 18 and 20 weeks of age were assigned to three groups (n=8) with similar mean body weights: the control (C) group fasted for 6 hours, the 12 hours fasting group (F12), and the 24 hours fasting group (F24). During the fasting period, mice had free access to water and the 12 h fasting period was over-night. After fasting blood glucose concentrations were measured and plasma and tissue samples were collected. Lithium heparin coated tubes (Sarstedt, Nümbrecht, Germany) were used for blood collection.

OGTT

Prior to the experiment, 24 mice were fasted for 6 hours and assigned to four groups (n=6-11) with similar mean body weight. Male mice between 14 to 18 weeks old were gavaged with 300 µl of a 20% glucose solution (B.Braun Melsungen AG, Melsungen, Germany; groups: 30 min, 60 min, 120 min) or with water (control). Blood glucose concentrations were measured and plasma and tissue samples were collected after 0, 30, 60, and 120 minutes (n=6). EDTA coated tubes (Sarstedt, Nümbrecht, Germany) were used for blood collection.

4.3. Human Metabolome-study

4.3.1. Subjects

The Human Metabolome (HuMet)-study was conducted in the Human study Center of the Else Kröner-Fresenius-Center for Nutritional Medicine and was previously described [98]. In total 15 healthy male volunteers with a narrow BMI (23.1 ± 1.76 kg/m²) and age range (27.8 ± 2.98 years) were recruited after a medical entry examination including determination of standard clinical (blood pressure, blood lipids

etc.) and anthropometric (BMI, waist-to-hip-ratio *etc.*) parameters. In addition, resting metabolic rate was determined using indirect calorimetry (Deltatrac metabolic monitor; Datex-Ohmeda, Helsinki, Finland). Body composition was measured by dual energy X-ray absorptiometry (Explorer bone densitometer, Hologic Inc., Bedford, MA, USA). The individual anaerobic threshold was assessed by an incremental bicycle ergometer test. The test started at 50 W and was performed at the Center for Prevention and Sports Medicine (Technische Universität München). Volunteers were included, when they did not show any abnormalities regarding the standard clinical chemistry and were free of any medication. The ethical committee of the Technische Universität München (#2087/08) approved the study protocol which corresponded with the Declaration of Helsinki.

4.3.2. Study design

Over the whole study period of four days, subjects underwent five metabolic challenges. Figure 36 displays the study design with time-scheduled sample-collection. For ethical reasons, the study was split into two test periods (two days and two nights each) separated by at least four weeks. Subjects were asked to neither consume any alcohol nor undertake strenuous physical exercise 24 hours prior to each test period. Already the evening before each test period, subjects moved into their room at the study center, received a standardized balanced meal at 7.00 p.m. and stayed inside the center throughout the test period to minimize the environmental effects. Gray lines in Figure 36 indicates additional standardized meals, that subjects consumed during the study (day 2 at 8 a.m., day 3 at 12 p.m., day 3 at 7 p.m.; Fresubin® Energy Drink chocolate, Fresenius Kabi AG, Bad Homburg, Germany). For detailed composition of the standardized meal see also Table 13. The total energy intake of each subject per test day was aligned to the individuals' resting metabolic rate and multiplied by the factor 1.3 for low physical activity. Each meal delivered 1/3 of the daily requirement. Sampling occurred at predefined points of time and baseline samples of each challenge test (= 0 min) were taken shortly before.

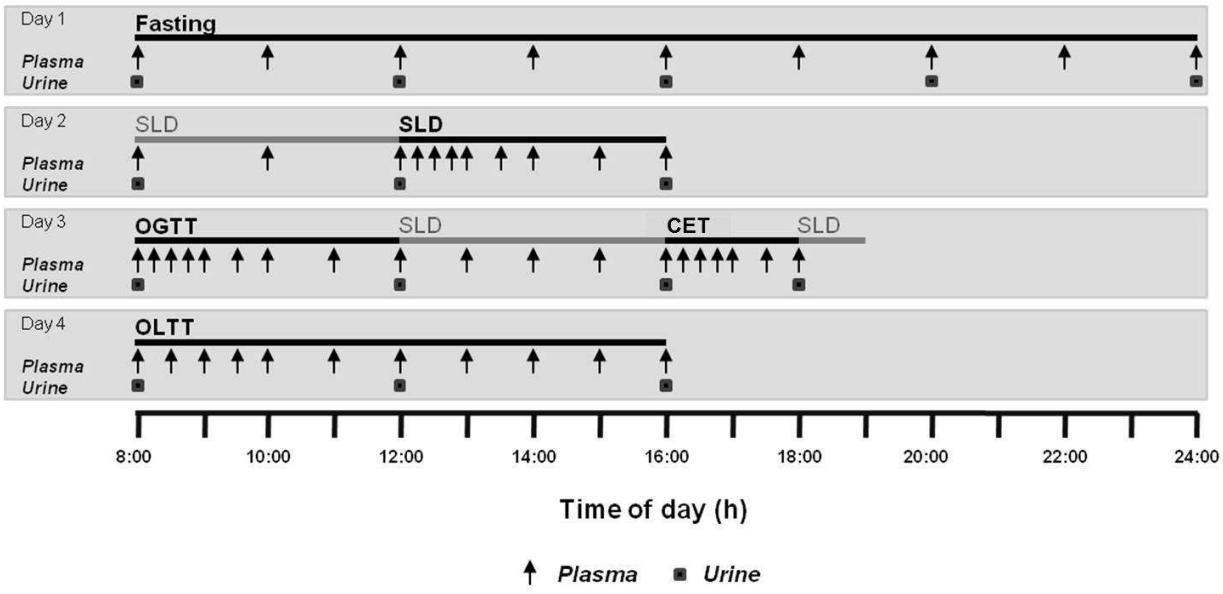

Figure 36: Study design of the HuMet-study
Abbreviations: Fasting, 36 hours fasting challenge; SLD, standard liquid diet; OGTT, oral glucose tolerance test; CET, cycling ergometer test; OLTT, oral lipid tolerance test. Adapted from [98].

4.3.3. Challenges

Fasting. After an over-night fasting period the first blood sampling took place at 8 a.m. on day 1 and was defined as point of time t = 0. Subjects continued fasting for additional 24 hours during which they were allowed to drink maximum 2.7 litres of mineral water according to a pre-defined drinking schedule. From 8 a.m. to 12 p.m., every second hour, blood was collected and again the next morning at 8 a.m. (day 2) to complete the 36 hours fasting period. Urine was sampled every four hours from 8 a.m. to 12 p.m. and after 36 hours of fasting. Collecting time-points for the fasting were 1 to 10.

Standard liquid diet (SLD). At 12 p.m. on day 2 the volunteers received a defined commercial fibre-free formula drink (Fresubin® Energy Drink chocolate, 50% carbohydrates, 35% fat, 15% protein, Fresenius Kabi AG, Bad Homburg, Germany) (Table 13). They were asked to ingest the drink within 5 minutes. As mentioned above the total amount of energy (in kcal) was 1/3 of the calculated individual daily requirements. Blood sampling took place before (t = 0 min) and after intake of the SLD at t = 15, 30, 45, 60, 90, 120, 180 and 240 minutes. Urine was collected at t = 0 min and 120 min after SLD ingestion. Collecting time-points for the SLD were 12 to 20.

OGTT. After an over-night fasting period, an OGTT was performed at 8 a.m. with 300 ml of a glucose solution (75 g glucose, Dextro O.G.T., Roche Diagnostics, Mannheim, Germany) on day 3. Volunteers had to finish drinking within 3 minutes. Blood samples were collected as in the SLD challenge at t = 0, 15, 30, 45, 60, 90, 120, 180 and 240 minutes. Urine samples were collected at t = 0 min and after the challenge (240 min). Collecting time-points for the OGTT were 21 to 29.

Cycling ergometer test (CET). On day 3 in the afternoon, volunteers performed a PAT by a 30 minutes bicycle ergometer training at their individual anaerobic threshold. Blood sampling took place before (t = 0 min), during (at t = 15, 30 min) and after (at t = 45, 60, 90 and 120 min) the cycling ergometer test. Urine was collected at 0 and 120 min. Collecting time-points for the CET were 33 to 39.

Table 13: Nutrient composition of the test meals of HuMet-study
Standardized liquid diet (SLD) and the oral lipid tolerance test (OLTT)

	SLD		OLTT	
	per 100 ml	**per subject**	**per 100 ml**	**per subject**
Volume (ml)	100	482 ± 78.6	100	415 ± 24.4
Energy (kJ)	630	3041 ± 494.2	935.5	3877 ± 223.1
Energy (kcal)	150	724 ± 117.7	225	932.4 ± 53.8
% protein	15	15	7.5	7.5
% carbohydrates	50	50	25.1	25.1
% fat	35	35	67.4	67.45
Protein (g)	5.6	27 ± 4.26	4.2	17.4 ± 1.2
Carbohydrates (g)	18.8	90.8 ± 14.7	14.1	58.4 ± 3.5
Fat (g)	5.8	28 ± 4.6	16.85	69 ± 3.9
Saturated FA (g)	0.5	2.4 ± 0.4	1.7	7.1 ± 0.4
Mono-unsaturated FA (g)	3.7	17.9 ± 3.1	10.38	43 ± 2.3
Poly-unsaturated FA (g)	1.6	7.7 ± 1.2	4.78	19.8 ± 1.2
Water (ml)	78	353 ± 107.7	58.5	242.4 ± 13.9

Abbreviations: FA, fatty acids.

Oral lipid tolerance test (OLTT). For the OLTT, which took place on day 4 at 8 a.m. after an over-night fast, each volunteer received 35 g fat per m² body surface area. Therefore, the amount of liquid meal was calculated and consisted of a mixture of 3 parts Fresubin® Energy Drink chocolate (Fresenius Kabi AG, Bad Homburg, Germany) and 1 part of a fat emulsion (Calogen®, Nutricia, Zoetemeer, Netherlands) (Table 13). Again, volunteers were asked to finish within 5 minutes and plasma sample collection took place at t = 0, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 minutes after the ingestion. Urine was collected at 0, 240 and 480 min. Collecting time-points for the OLTT were 40 to 50.

4.3.4. Sampling

Plasma and urine samples were aliquoted directly after collection, frozen on dry ice and stored at -80°C until analysis.

Plasma. Throughout the study blood sampling was carried out using a venous cannula (18 G 1 3/4, Vasofix Braunüle, Braun, Germany) inserted into an antecubital vein. Blood was collected into EDTA K_2-Gel tubes (Sarstedt, Nümbrecht, Germany), mixed and directly centrifuged (3,000 x g, 10 min, RT).

Urine. Spot urine samples were collected into 125 ml-PP-beakers (Ratiolab, Dreieich, Germany).

4.4. Metabolomics techniques

4.4.1. Amino acid analysis via LC-MS/MS

iTRAQ. For quantification of amino acids in plasma, urine and tissues the derivatization method with iTRAQ® (AA45/32™ Phys REAG Kit, Applied Biosystems, Foster City, USA) was used as described previously [82]. For measurement of tissue amino acids, small pieces of tissue were homogenized in liquid nitrogen using mortar and pestle. 100 mg of the homogenized tissue were dissolved in 150 µl MeOH/H_2O (50:50, v/v), vortexed and centrifuged (10000 x g) for 10 min at 4°C. As described previously [170], sample preparation was performed according to the manufacturer's instructions with slight modifications. For tissues, 40 µl of the supernatant were used, while for plasma or urine samples 40 µl of the bio fluids were added to 10 µl of 10% sulfosalicylic acid containing norleucine (400 pmol/µl) for precipitation of proteins. The samples were vortexed and centrifuged for 2 min at 10000 x g. 10 µl of the supernatant was diluted with 40 µl labeling buffer (borate buffer, pH 8.5, containing 20 pmol/µl norvaline). Then 10 µl of the dilutions were transferred to new tubes and 5 µl iTRAQ® 115 reagent were added to each tube. Samples were mixed, vortexed and incubated for 30 min at room temperature. Afterwards, 5 µl hydroxylamine (1.2%) were added to the samples and the samples were dried in a centrifugal vacuum concentrator (SPD 111V SpeedVac, Thermo Savant, Germany) for about 45 min. Samples were resolved adding 32 µl 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 128 µl mobile phase A (0.1% formic acid and 0.01%

heptafluorobutyric acid solved in water). Afterwards, 10 µl of the prepared samples were automatically injected (autosampler HTC PAL, CTC Analytics AG, Zwingen, Switzerland) and the derivatized 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/MS/MS system (Agilent Technologies 1200 Series, Agilent Technologies GmbH, Germany). The mobile phase consisted of 0.1% formic acid and 0.01% heptafluorobutyric acid solved in water (mobile phase A) or acetonitrile (mobile phase B) respectively. Flow rate was 800 µl per min. Mass analysis was performed using the 3200QTRAP LC/MS/MS system (Applied Biosystems, Foster City, USA) operating in a multiple reaction-monitoring mode. The Analyst® 1.5 Software (Applied Biosystems, Foster City, USA) was used to quantify the amino acid concentrations. Both spiked amino acids norleucine and norvaline were used for quality control either for workflow efficiency or for labeling efficiency. In case of tissue samples, amino acid concentrations were normalized to protein concentrations obtained via Bradford assay [172]. Urinary amino acid concentrations were normalized to osmolarity.

aTRAQ. Analysis was performed with the aTRAQ^{TM} Reagent Kit 200 Assay (ABSciex, Foster City, USA). In contrast to the iTRAQ-labeling method, sample amino acids and derivatives were derivatized with an aTRAQ-reagent (M = 121 g/mol) and an aTRAQ-labeled amino acid standard mix (M = 113 g/mol) was added. Methanol containing 0.1% formic acid and 0.01% heptafluorobutyric acid was used as mobile phase B.

4.4.2. Untargeted GC-MS analysis

Sample preparation

Plasma metabolites were extracted using four volumes of ice-cold methanol. Paracetamol was added as internal standard (0.5 mg/ml) to the extraction buffer. Samples were shaken for 10 min at 4°C followed by centrifugation (14000 x g, 4°C) for 10 minutes. The supernatant was transferred to a new tube and dried using a centrifugal vacuum concentrator (SPD 111V SpeedVac, Thermo Savant, Germany).

GC-MS analysis

Derivatization. As described previously [173], dried samples were derivatized for metabolite analysis via GC-MS in a two-step procedure. First, carbonyl moieties were protected via methoximation with 20 µl of 40 mg/ml methoxyamine hydrochloride in pyridine (90 min, 30°C). Afterwards, acidic protons were derivatized by adding 32 µl MSTFA (N-methyl-N-trimethylsilyltrifluoroacetamide) and incubating the samples for 60 min at 37°C. For determination of retention time index, 1 µl of the retention time index marker cocktail (n-alkanes ranging from C10 to C36, each 200 µg/ml in pyridine) was added to each sample.

Hardware system. For metabolite profiling, an HP Agilent 7890 gas chromatograph was used to perform GC analysis and was coupled to an Agilent 5975 Quadropole mass spectrometer (Agilent Technologies, Böblingen, Germany) for mass determination. Samples were injected by a volume of 1 µl using a splitless mode and separation was performed on a VF-5ms capillary column, 30 m long (0.25 mm inner diameter, 25 µm film thickness (VARIAN, Palo Alto, USA)) at a constant helium flow of 1 ml per minute. Temperature started isocratic with 1 min at 70°C and was progressively ramped at 10°C/min up to 330°C which was held for 8 min. Mass analysis was performed by scan rates of 5 s^{-1} and mass ranges of 70 to 600 Da.

Data processing. The MetaboliteDetector software (version 2.06) was used for the processing of all chromatograms. This included automated baseline correction, peak finding, area calculation, library search and deconvolution of all chromatogram mass spectra and mass-spectral correction for co-eluting metabolites. Retention indices (RI) were calculated and suitable fragment mass-to-charge ratios for selective quantification were identified. In order to identify metabolites RI and defined spectra, the Golm Metabolome Database and an in-house library were used. For peak area calculation selected fragment ions specific for each individual metabolite were used. Finally, each compound was normalized by the peak area of the internal standard and the obtained relative response ratios could be compared without knowledge of absolute compound concentrations.

As MetaboliteDetector software supports unbiased profiling, novel and unexpected compounds were discovered. For an experimental setting all peaks determined in each chromatogram were aligned. In total, after alignment 600 to 900 deconvoluted

peaks per chromatogram were filtered via 70% reproducibility and exclusion of systemic peaks. The procedure allowed us to include and identify around 110 metabolites for plasma in the analysis, from which derivatives and adducts have to be summed up to biological compounds.

4.4.3. GC-MS for hepatic fatty acid analysis

Sample preparation and analysis via GC-MS was done at the Institute of Food and Health at University College Dublin. As described previously [170], for fatty acid extraction of liver tissue, approximately 100 mg of frozen tissue was homogenized in 600 µl of a 1:2 mixture of chloroform:methanol. Samples were sonicated and an equal volume of a 1:1 mixture of chloroform:water was added. Samples were mixed, incubated for 15 min and centrifuged. The aqueous layer was discarded. Twenty µl of 2 mg/ml pentadecanoic acid (C15:0) were added to the organic layer as an internal standard. Afterwards, samples were dried under nitrogen gas followed by derivatization via methylation. Therefore, 1 ml methanolic BF3 was added and samples were incubated (100°C, 45 min). Samples were again dried under nitrogen gas and stored until analysis.

Samples were resuspended by adding 200 µl of hexane. Analysis was performed on an Agilent 7890A GC coupled with a 5975C MS with an Agilent HP-5ms 30 m x 250 µm x 0.25 µm column (Agilent, Santa Clara, USA). Samples (1 µl) were injected in splitless mode. The initial oven temperature of 70°C was raised to 220°C at 5°C/min, held for 20 min, and then raised to 320°C at 20°C/min. Helium was used as the carrier gas with a flow-rate of 1.2 ml/min. For calibration, peak areas were compared to a known standard (Supelco 37 compound mix, Supelco, Poole, UK) using Agilent Chemstation MSD E.02.00.493. and to NIST Library 2.0 (2005). Automatic peak detection was performed with Agilent Chemstation MSD (Agilent, Santa Clara, USA). Mass spectral deconvolution was carried out with AMDIS version 2.65. All peaks with a signal-to-noise ratio lower than 30 were rejected. To obtain accurate peak areas for the internal standard and specific peaks/compounds, one quant mass for each peak was specified as a target. Three masses were used as qualifiers. Each data file was manually analysed to detect false positives/negatives using Agilent Chemstation. The concentrations reported here were standardized to protein concentrations.

4.4.4. ^{1}H NMR spectroscopy

Preparation and ^{1}H NMR analysis of plasma samples was done at the Institute of Food and Health at University College Dublin as described previously [174]. In brief, plasma was prepared by adding 250 µl D_2O and 10 µl trimethylsilyl to 300 µl plasma. Subsequent spectra were acquired with a 600-MHz Varian NMR spectrometer (Varian Limited, Oxford, United Kingdom) by using a Carr-Purcell-Meiboom-Gill pulse sequence with 32-K data points and 64 scans. Chenonmx software (version 6; Chenonmx, Edmonton, Canada) was used to process manually all ^{1}H NMR plasma spectra. All spectra were referenced to trimethylsilyl and then phase and baseline corrected.

Spectra were integrated into bins consisting of spectral regions of 0.04 ppm using Chenonmx software. Bins from the water region (4.0-6.0 ppm) were excluded, and data were normalized to the sum of the spectral integral. Using libraries of pure metabolites developed in house and the Chenomx database library, metabolites were identified.

4.4.5. Analysis of acylcarnitine species

Ten µl of plasma were analyzed using the Absolute*IDQ* kit p150 (Biocrates Life Sciences AG, Innsbruck, Austria). Sample preparation was done according to the manufacturers' instructions as it was described previously [11, 98]. By flow injection, analysis samples were delivered to the mass spectrometer and mass analysis was performed using the 3200 LC/MS/MS system (Applied Biosystems, Foster City, USA) and multiple reaction-monitoring pairs. Protein concentration determined using the Bradford assay [172] was used to normalize metabolite concentrations. The determination of acylcarnitines in mouse plasma was performed using an adapted in-house method.

4.5. Analysis of osmolarity and creatinine in urine

Creatinine was determined via the Creatinin Liquicolor Jaffè assay (Rolf Greiner BioChemica, Flacht, Germany) and osmolarity was measured using the Semi Micro Osmometer (Knauer, Berlin, Germany).

4.6. Everted gut sacs

To investigate the intestinal citrulline production, the everted gut sacs technique in combination with $^{13}C^{15}N$-labeled arginine (labeling rate for ^{13}C: 98%, and for ^{15}N: 98%; Silantes GmbH, Munich, Germany) was used. This enabled to distinguish between citrulline production via the arginase pathway and citrulline production via NO-synthase (Figure 37).

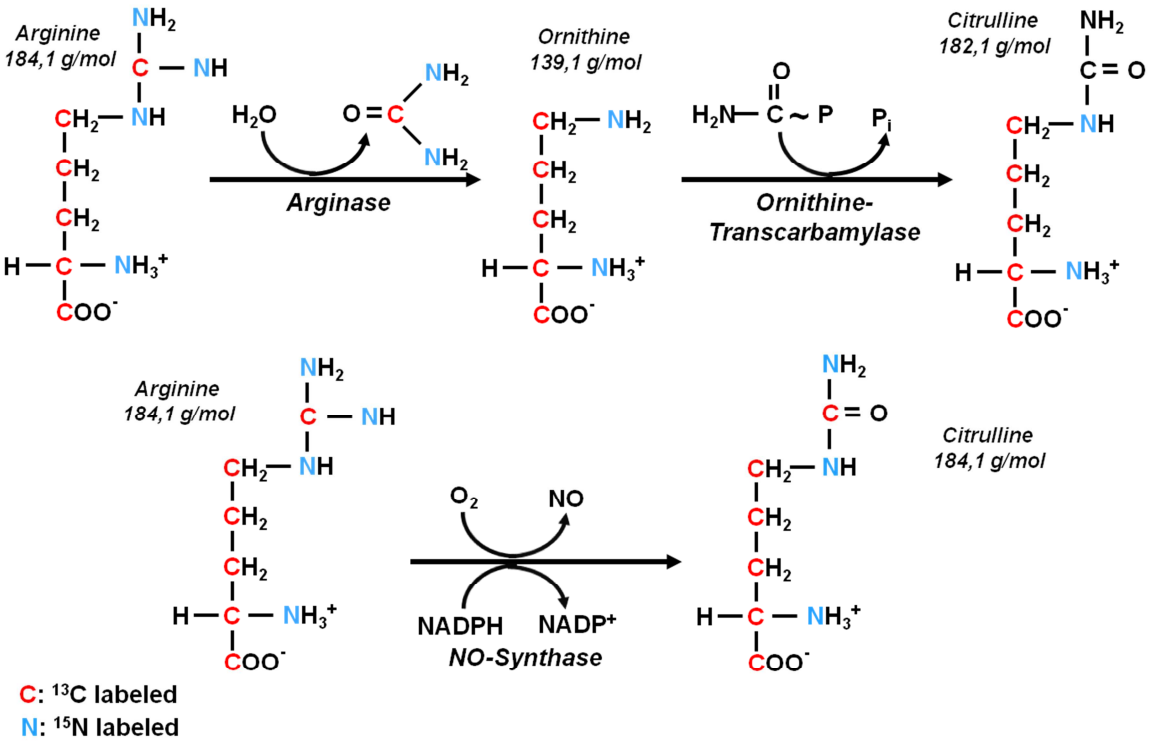

Figure 37: Possible pathways of arginine to citrulline conversion in small intestine
(A) Arginine is either metabolized via arginase to ornithine and further converted to citrulline via ornithine-transcarbamylase or (B) arginine is directly converted to citrulline via NO-synthase. Both ways of arginine conversion lead to a production of citrulline, however the molecular mass of citrulline is different depending on the pathway and thus can be detected via mass-spectrometry.

The whole small intestine was taken out and mesenteric fat was thoroughly removed, as well as the first 7 cm of the small intestine. The remaining part was washed and rinsed with ice-cold Krebs buffer, pH 7.4 (119 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, and additional 1% HEPES) and subsequently everted. The whole segment was used to cut 2 cm pieces beginning at the proximal site. Per mouse, 2 to 4 sacs were prepared. Each sac was filled with approximately 200 µl of Krebs buffer. Sacs were incubated at 37°C for 30 min on a

shaker in 2 ml Krebs buffer solution containing 0.1 mM $^{13}C^{15}N$-labeled arginine and 2 mM glutamine. Glutamine was shown to enhance intestinal arginine to citrulline conversion [44]. As a control the arginase specific inhibitor N-ω-hydroxy-L-norarginine (Bachem Holding AG, Bubendorf, Switzerland) [175] (0.1 mM) was used. During the incubation, Krebs buffer was oxygenated with carbogen (5% CO_2/95% O_2). After incubation, the sacs were washed in Krebs buffer and carefully opened. The serosal fluid and the incubation buffer were collected in a tube, aliquoted and stored at -80°C until analysis. Via the iTRAQ-labeling method (see Amino acid analysis via LC-MS/MS), the conversion of labeled arginine to citrulline was determined using 40 µl of the collected fluids. Sacs were dried over-night at 60°C and digested with 1 M NaOH at 37°C. Intestinal ornithine and citrulline release were standardized with protein concentrations which were determined via Bradford assay [172].

4.7. Statistical analysis

All statistical analysis was performed using the R statistical software version 2.12.2 (http://www.r-project.org) and data are presented as mean ± SD unless stated otherwise. For the analysis of the HuMet-study missing values were replaced by the mean of all samples of a specific time-point for each parameter. Time-dependent changes in plasma observed in the challenges of the HuMet-study were analyzed by repeated-measurement ANOVA. For post hoc testing, linear contrasts were used for pair-wise comparison followed by Tukey using the function *ptukey*. For mouse studies, statistical testing was performed either via two-way, one-way ANOVA or unpaired Student's t-test. *P*-values were adjusted applying the *p.adjust* function and Benjamini-Hochberg correction unless stated otherwise. Pair-wise comparison was performed by Tukey post-hoc test. Significance was assumed when *P-value* < 0.05. The packages *lattice*, latticeExtra, RColorBrewer, *plyr, reshape, scatterplot3d, Hmisc* and *qgraph* were used for data transformation, creating graphs, PCA, hierarchical clustering, correlation and correlation network analysis. For correlation network analysis the 'spring' algorithm as layout within the *qgraph* package was applied.

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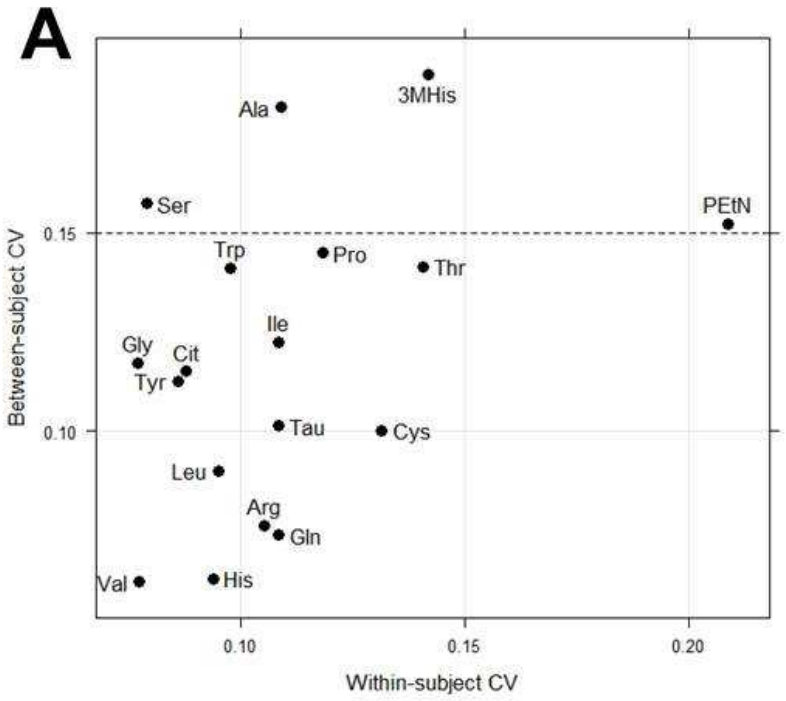
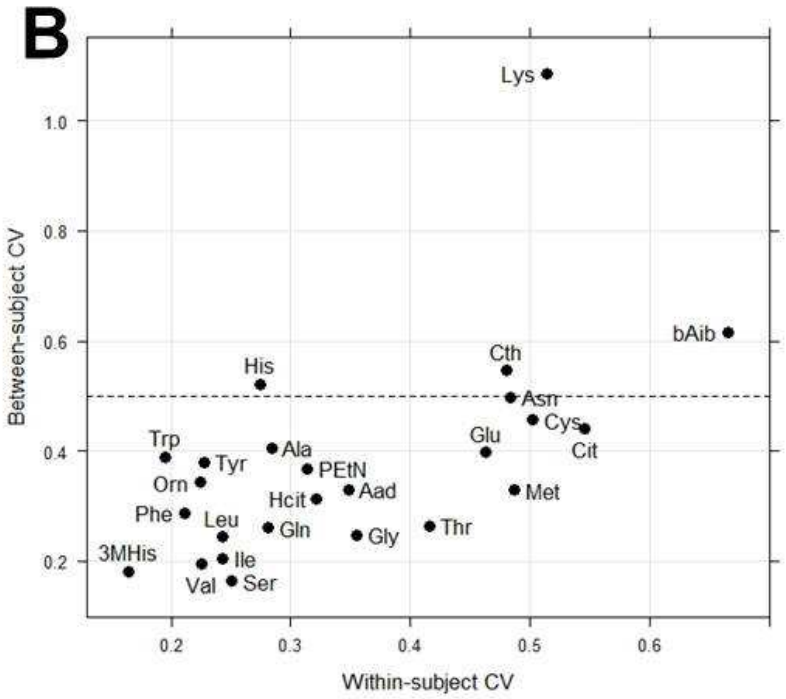
Appendix

A Supplementary data

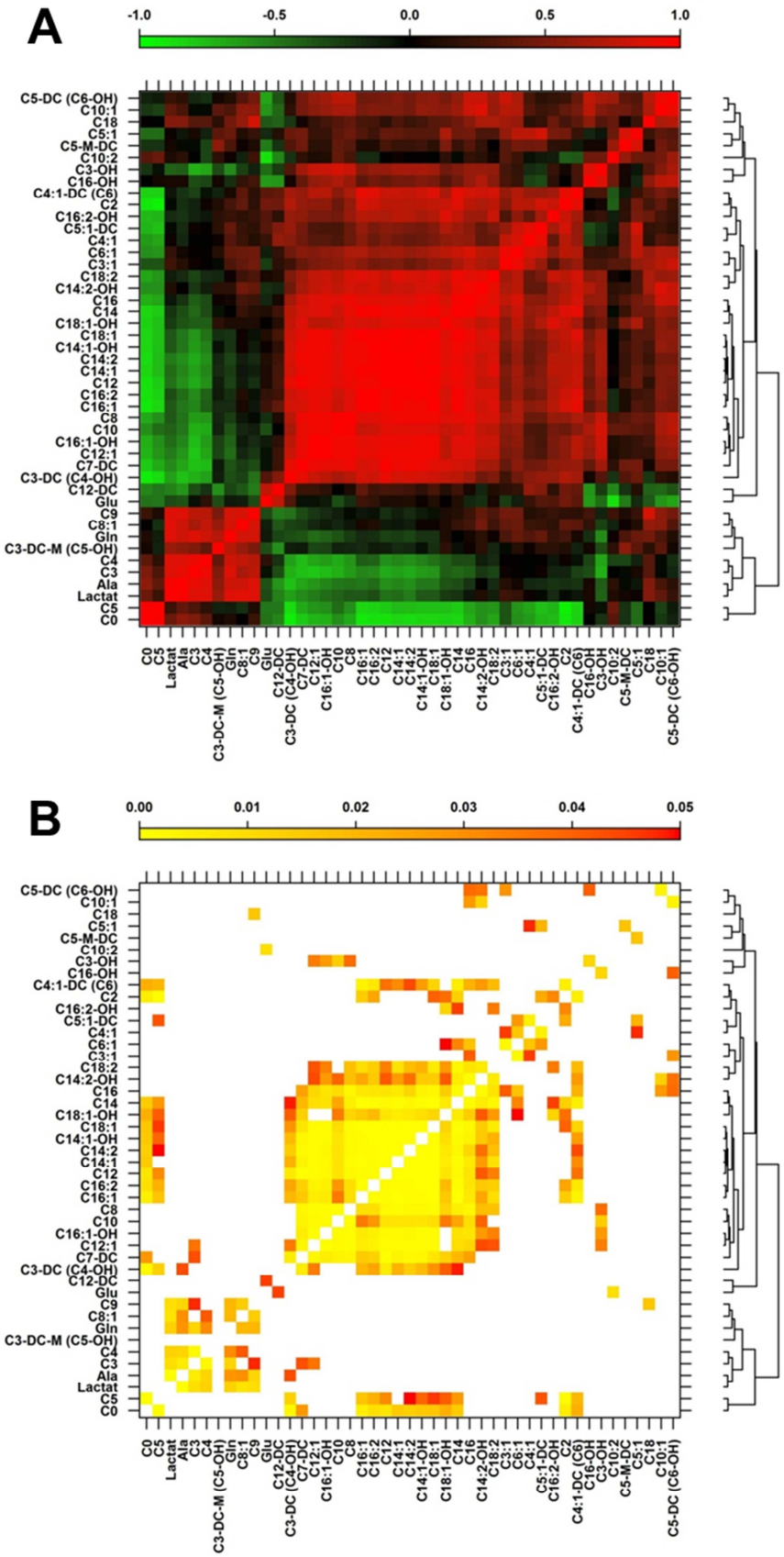
Suppl. Figure 38: Subject variability in plasma (A) and urinary (B) amino acid concentrations
Between subject coefficient of variance was plotted against within-subject coefficient of variance. Dashed black line indicates a between-subject coefficient of variance equal to 0.15 for (A) and 0.5 for (B).





Suppl. Figure 39: Pearson correlation matrix of acylcarnitines and selected amino acids during CET

Correlations were calculated between mean concentrations of plasma alanine, glutamine, glutamate and lactate and acylcarnitine species in plasma (A). Corresponding matrix displaying *p*-values of the correlations (B).



Suppl. Table 14: Plasma amino acids of fasting challenge (HuMet-study)

Volunteers (n=15) were fasted for 36 hours and amino acids were determined via LC-MS/MS.

	Time-point									
	1	2	3	4	5	6	7	8	9	10
Amino acids	Time (minutes)									
(µmol/l)	**0**	**120**	**240**	**360**	**480**	**600**	**720**	**840**	**960**	**1440**
α-aminobutyrate*	24.27 ± 4.90	24.37 ± 4.81	24.07 ± 3.62	25.58 ± 4.82	26.92 ± 5.17	29.91 ± 6.55	34.21 ± 5.69	37.73 ± 6.66	40.80 ± 7.64	52.03 ± 9.20
Alanine*	398.40 ± 82.32	411.80 ± 80.87	404.79 ± 77.14	343.62 ± 49.82	323.53 ± 40.28	333.40 ± 42.82	345.47 ± 53.27	312.47 ± 61.28	307.93 ± 77.93	346.20 ± 67.08
Arginine*	85.38 ± 11.44	84.03 ± 10.37	86.56 ± 10.77	80.78 ± 9.71	79.45 ± 11.35	82.83 ± 12.57	86.04 ± 10.28	78.21 ± 12.68	76.87 ± 13.84	75.69 ± 11.14
Asparagine	74.37 ± 6.89	72.17 ± 7.62	75.71 ± 9.78	70.12 ± 6.84	70.94 ± 6.28	71.39 ± 7.83	72.79 ± 7.35	69.82 ± 8.72	70.42 ± 7.12	69.09 ± 6.40
Citrulline*	34.54 ± 4.93	31.74 ± 5.00	30.65 ± 3.60	28.95 ± 4.85	29.95 ± 4.61	29.13 ± 4.99	29.17 ± 3.94	29.43 ± 4.52	31.15 ± 5.89	30.13 ± 4.33
Cystine	103.19 ± 16.28	105.43 ± 23.24	97.51 ± 12.63	106.98 ± 19.71	105.35 ± 18.48	102.60 ± 18.30	108.16 ± 13.96	109.07 ± 19.13	112.47 ± 17.57	109.05 ± 19.63
Ethanolamine*	8.27 ± 1.08	8.41 ± 1.37	8.62 ± 1.30	8.93 ± 1.11	9.24 ± 0.84	9.60 ± 1.17	9.78 ± 0.88	8.70 ± 1.06	8.86 ± 1.41	11.73 ± 2.82
Glutamate*	37.21 ± 14.44	22.32 ± 4.74	25.02 ± 10.68	28.03 ± 11.75	22.37 ± 7.38	21.17 ± 7.89	20.95 ± 9.78	26.99 ± 15.75	27.27 ± 13.01	26.47 ± 14.28
Glutamine	674.93 ± 115.40	686.80 ± 70.24	713.86 ± 129.20	666.69 ± 52.18	687.87 ± 74.02	693.00 ± 90.94	712.13 ± 68.39	646.87 ± 71.52	650.40 ± 68.25	656.53 ± 45.79
Glycine*	258.93 ± 39.29	257.33 ± 33.69	254.64 ± 42.66	244.77 ± 23.37	229.80 ± 37.71	239.00 ± 30.39	233.27 ± 34.16	224.13 ± 32.41	218.93 ± 39.15	216.60 ± 43.52
Histidine	92.65 ± 12.63	89.19 ± 9.43	92.80 ± 10.46	84.59 ± 9.25	87.00 ± 10.20	89.87 ± 15.30	94.89 ± 9.84	90.07 ± 11.59	92.54 ± 12.70	95.61 ± 7.71
Hydroxyproline*	11.01 ± 2.42	10.92 ± 3.26	10.86 ± 3.06	9.22 ± 2.24	9.14 ± 2.03	8.83 ± 2.19	8.70 ± 1.70	7.90 ± 1.60	7.48 ± 1.54	7.05 ± 1.49
Isoleucine*	75.69 ± 11.53	69.09 ± 12.17	70.42 ± 12.06	68.23 ± 9.92	72.42 ± 6.28	81.95 ± 11.84	91.36 ± 11.28	96.78 ± 10.56	104.87 ± 12.24	130.11 ± 26.44
Leucine*	150.20 ± 16.76	141.93 ± 14.77	146.36 ± 16.14	145.00 ± 12.83	155.47 ± 13.35	172.93 ± 22.43	190.47 ± 19.54	195.53 ± 24.89	210.27 ± 23.81	263.53 ± 53.43
Lysine	179.80 ± 18.32	186.40 ± 19.22	190.07 ± 20.69	179.54 ± 18.46	179.67 ± 16.99	181.47 ± 23.12	189.40 ± 22.56	177.80 ± 21.22	179.87 ± 27.54	187.73 ± 30.61
Ornithine*	55.13 ± 9.15	52.77 ± 7.71	53.03 ± 6.45	51.20 ± 7.83	49.43 ± 6.94	48.82 ± 6.62	50.30 ± 6.16	50.87 ± 7.32	53.99 ± 9.47	51.30 ± 7.85
P-ethanolamine*	3.25 ± 0.51	3.12 ± 0.73	3.26 ± 0.50	3.28 ± 0.69	3.39 ± 0.45	3.34 ± 0.63	3.61 ± 0.65	3.32 ± 0.70	3.44 ± 0.80	4.05 ± 1.18
Phenylalanine*	60.26 ± 7.20	57.48 ± 6.10	59.61 ± 7.34	57.57 ± 4.51	60.81 ± 5.42	63.35 ± 6.21	66.87 ± 3.34	64.23 ± 3.38	64.22 ± 4.90	65.54 ± 4.99
Proline*	234.73 ± 35.14	217.53 ± 35.91	208.07 ± 32.74	183.00 ± 31.62	181.00 ± 25.78	183.20 ± 29.85	185.33 ± 26.04	171.20 ± 22.38	172.20 ± 26.09	172.33 ± 26.12
Sarcosine	4.76 ± 1.01	4.89 ± 1.10	4.67 ± 0.94	4.27 ± 1.02	4.59 ± 1.02	4.64 ± 1.13	4.30 ± 0.74	4.31 ± 0.74	4.46 ± 0.75	4.05 ± 1.03
Serine	121.22 ± 23.54	116.57 ± 24.93	118.62 ± 25.71	118.65 ± 22.42	116.59 ± 18.04	118.92 ± 22.62	120.75 ± 19.95	116.34 ± 15.66	115.69 ± 18.04	114.84 ± 17.50
Taurine*	54.43 ± 6.24	53.63 ± 5.45	53.36 ± 4.09	53.44 ± 6.79	55.10 ± 5.44	55.57 ± 6.46	58.21 ± 6.53	56.50 ± 6.08	60.90 ± 6.40	68.25 ± 9.48
Threonine*	145.06 ± 31.28	140.17 ± 33.14	142.64 ± 31.25	126.05 ± 28.09	125.22 ± 23.78	125.84 ± 24.25	124.93 ± 17.72	117.45 ± 15.43	110.30 ± 15.43	112.36 ± 16.98
Tryptophan*	60.63 ± 8.98	55.31 ± 8.97	55.27 ± 5.74	52.21 ± 6.27	51.27 ± 3.64	52.47 ± 4.97	53.53 ± 7.41	52.10 ± 6.67	50.74 ± 9.72	44.96 ± 14.23
Tyrosine*	65.65 ± 8.37	61.91 ± 9.38	61.29 ± 9.75	58.12 ± 6.61	59.45 ± 11.00	59.24 ± 9.99	62.25 ± 7.24	58.89 ± 6.86	57.95 ± 6.47	60.65 ± 4.64
Valine*	278.20 ± 26.42	266.07 ± 25.53	266.07 ± 28.44	257.08 ± 24.25	264.07 ± 21.17	276.00 ± 27.29	296.13 ± 26.69	302.20 ± 28.91	318.33 ± 32.04	382.47 ± 53.23
1-M-Histidine*	15.85 ± 3.71	13.64 ± 3.37	11.79 ± 3.30	11.36 ± 2.92	10.61 ± 2.66	9.96 ± 3.67	8.86 ± 2.54	8.00 ± 2.13	8.11 ± 2.40	5.73 ± 1.65
3-M-Histidine	5.47 ± 1.46	5.10 ± 1.26	5.16 ± 1.16	5.24 ± 1.11	5.18 ± 1.24	5.37 ± 1.41	5.24 ± 1.28	5.55 ± 1.46	5.29 ± 1.39	5.69 ± 1.49
Sum*	3313.51 ± 235.62	3250.15 ± 243.88	3274.79 ± 310.44	3072.50 ± 218.07	3075.81 ± 210.12	3153.79 ± 274.10	3267.11 ± 172.10	3122.49 ± 180.45	3165.77 ± 243.17	3369.78 ± 186.22

Data are presented as mean as mean ± SD. **P*-value < 0.05 obtained by repeated-measurement ANOVA for time-effects and adjusted with Benjamini-Hochberg correction.

Suppl. Table 15: Plasma amino acids of OGTT (HuMet-study)

Volunteers (n=15) were challenged with an oral glucose tolerance test (75 g glucose) and amino acids were determined via LC-MS/MS.

	Time-point								
	21	22	23	24	25	26	27	28	29
Amino acids (µmol/l)	Time (minutes)								
	0	15	30	45	60	90	120	180	240
α-aminobutyrate*	25.15 ± 5.99	25.49 ± 5.36	23.58 ± 5.11	22.03 ± 5.57	21.03 ± 4.95	20.03 ± 5.10	19.15 ± 4.55	18.64 ± 4.09	20.07 ± 4.82
Alanine	374.13 ± 65.43	364.07 ± 69.74	369.07 ± 68.46	375.07 ± 68.29	382.60 ± 57.75	382.80 ± 45.64	380.46 ± 47.62	375.36 ± 43.75	388.93 ± 45.20
Arginine*	87.47 ± 10.58	86.15 ± 11.87	79.95 ± 11.07	77.73 ± 13.60	73.51 ± 13.93	67.05 ± 10.92	64.55 ± 10.90	69.14 ± 11.09	72.31 ± 13.34
Asparagine*	65.97 ± 8.31	65.34 ± 8.45	61.27 ± 8.64	58.22 ± 7.45	55.97 ± 7.76	53.67 ± 7.55	55.24 ± 6.53	54.50 ± 6.15	61.55 ± 7.51
Citrulline*	34.03 ± 5.13	29.92 ± 5.01	27.19 ± 5.35	24.61 ± 5.21	22.47 ± 4.54	20.65 ± 3.71	19.23 ± 3.51	22.54 ± 4.57	26.08 ± 5.27
Cystine*	104.56 ± 21.12	104.36 ± 20.22	107.37 ± 15.57	101.72 ± 20.90	96.39 ± 15.13	89.14 ± 14.26	98.15 ± 12.83	94.61 ± 12.19	110.01 ± 15.79
Ethanolamine*	9.07 ± 1.61	8.40 ± 1.71	7.65 ± 1.44	8.26 ± 1.21	8.37 ± 1.11	8.00 ± 1.37	8.19 ± 1.44	8.98 ± 1.50	10.84 ± 2.89
Glutamate*	39.35 ± 17.88	42.29 ± 18.20	42.60 ± 16.13	36.73 ± 17.16	33.40 ± 18.08	40.41 ± 18.07	36.35 ± 15.06	31.44 ± 15.63	26.69 ± 11.29
Glutamine*	672.47 ± 92.33	630.60 ± 56.23	636.93 ± 57.23	622.93 ± 62.10	598.13 ± 59.35	584.93 ± 61.57	578.93 ± 56.03	591.71 ± 69.79	663.87 ± 93.46
Glycine*	256.27 ± 34.02	252.33 ± 31.16	238.20 ± 25.51	236.47 ± 35.20	228.47 ± 26.86	227.73 ± 37.10	223.87 ± 30.65	229.00 ± 29.50	230.53 ± 23.56
Histidine*	87.92 ± 9.68	87.81 ± 7.99	86.46 ± 7.63	84.45 ± 8.65	79.45 ± 8.10	75.93 ± 7.81	77.18 ± 7.30	79.85 ± 7.13	88.05 ± 7.71
Hydroxyproline*	11.83 ± 4.14	11.56 ± 3.54	11.02 ± 3.95	10.44 ± 3.49	10.14 ± 4.03	9.48 ± 3.53	9.56 ± 3.23	9.57 ± 2.80	10.56 ± 4.06
Isoleucine*	75.42 ± 10.86	74.13 ± 13.42	66.00 ± 10.62	59.36 ± 10.11	53.70 ± 9.00	46.07 ± 9.74	42.45 ± 8.56	42.25 ± 6.02	52.37 ± 7.31
Leucine*	147.53 ± 18.86	144.73 ± 24.01	131.32 ± 19.39	119.79 ± 17.29	109.33 ± 13.05	96.17 ± 15.54	91.95 ± 14.28	93.76 ± 13.29	111.70 ± 14.05
Lysine*	180.00 ± 20.93	177.00 ± 19.02	167.60 ± 25.42	162.07 ± 22.40	157.27 ± 22.27	151.47 ± 24.19	152.29 ± 15.61	157.79 ± 18.08	171.73 ± 19.02
Ornithine*	60.18 ± 11.12	59.93 ± 13.25	58.01 ± 10.68	52.28 ± 9.03	52.20 ± 10.72	48.27 ± 8.18	47.77 ± 8.64	46.99 ± 6.39	50.37 ± 8.81
P-ethanolamine*	2.98 ± 0.87	2.82 ± 1.01	2.64 ± 0.72	2.57 ± 0.78	2.53 ± 0.73	2.52 ± 0.66	2.78 ± 0.58	3.11 ± 0.87	3.52 ± 0.94
Phenylalanine*	58.64 ± 5.34	57.07 ± 6.01	52.80 ± 4.63	50.59 ± 5.43	49.77 ± 5.50	45.60 ± 5.76	44.53 ± 5.41	44.06 ± 2.89	49.17 ± 3.88
Proline*	207.47 ± 42.62	204.53 ± 45.63	192.73 ± 37.16	191.07 ± 44.89	179.07 ± 41.68	174.87 ± 44.50	172.18 ± 39.31	166.57 ± 31.20	184.40 ± 42.01
Sarcosine	4.43 ± 0.97	4.70 ± 0.98	4.34 ± 0.58	4.23 ± 0.68	4.09 ± 0.70	4.00 ± 0.79	4.39 ± 0.74	4.09 ± 0.77	4.68 ± 0.61
Serine*	121.49 ± 19.78	119.89 ± 19.95	113.44 ± 17.84	103.51 ± 16.98	103.29 ± 24.25	98.83 ± 17.07	98.78 ± 19.66	93.34 ± 16.93	105.73 ± 19.63
Taurine*	55.12 ± 9.82	54.63 ± 10.62	52.45 ± 9.09	52.46 ± 9.61	49.88 ± 9.16	52.41 ± 8.33	51.17 ± 7.52	55.06 ± 9.22	55.25 ± 7.69
Threonine*	135.64 ± 25.68	134.80 ± 23.82	125.29 ± 19.81	118.26 ± 20.85	114.15 ± 20.67	107.42 ± 23.63	105.99 ± 21.07	106.74 ± 17.27	117.77 ± 19.12
Tryptophan*	60.18 ± 10.23	57.09 ± 9.07	54.81 ± 8.25	53.13 ± 10.73	51.70 ± 8.00	48.18 ± 7.61	46.82 ± 6.50	45.51 ± 5.44	47.01 ± 6.03
Tyrosine*	62.89 ± 8.26	61.03 ± 9.67	55.93 ± 8.14	54.07 ± 9.51	51.17 ± 8.69	45.90 ± 8.09	44.06 ± 7.35	42.57 ± 7.68	50.88 ± 10.26
Valine*	280.53 ± 30.36	274.33 ± 35.05	260.40 ± 30.55	245.87 ± 25.53	236.33 ± 29.57	223.53 ± 30.46	216.62 ± 26.15	216.14 ± 19.41	232.27 ± 27.49
1-M-Histidine*	15.50 ± 4.34	15.47 ± 4.76	14.90 ± 4.21	13.97 ± 3.91	13.12 ± 3.65	12.70 ± 4.05	12.41 ± 3.49	12.24 ± 2.92	11.78 ± 3.33
3-M-Histidine*	5.67 ± 1.32	5.63 ± 1.32	5.25 ± 1.03	5.20 ± 1.20	5.07 ± 0.97	5.04 ± 1.07	4.99 ± 1.23	5.05 ± 1.09	5.08 ± 1.08
Sum*	3241.90 ± 272.71	3156.11 ± 234.70	3049.20 ± 228.60	2947.07 ± 288.99	2842.60 ± 230.80	2742.78 ± 245.65	2710.07 ± 199.22	2720.61 ± 181.23	2963.20 ± 213.77

Data are presented as mean as mean ± SD. **P*-value < 0.05 obtained by repeated-measurement ANOVA for time-effects and adjusted with Benjamini-Hochberg correction.

Suppl. Table 16: Plasma amino acids SLD (HuMet-study)

Volunteers (n=15) were challenged with a standard liquid diet (SLD) and amino acids were determined via LC-MS/MS.

	Time-point								
	12	13	14	15	16	17	18	19	20
Amino acids	Time (minutes)								
(µmol/l)	0	15	30	45	60	90	120	180	240
α-aminobutyrate*	39.31 ± 7.93	40.77 ± 8.07	42.19 ± 10.11	41.99 ± 8.12	40.80 ± 9.32	37.71 ± 7.27	36.28 ± 7.57	35.41 ± 7.42	35.61 ± 8.45
Alanine*	414.60 ± 55.93	393.60 ± 52.12	398.20 ± 57.54	430.86 ± 68.52	445.27 ± 67.84	471.33 ± 61.31	491.53 ± 43.33	446.73 ± 30.06	424.60 ± 37.14
Arginine*	54.68 ± 14.47	61.49 ± 16.14	65.11 ± 17.82	59.49 ± 13.91	58.73 ± 16.18	64.69 ± 12.78	60.99 ± 13.94	50.81 ± 12.89	49.39 ± 9.99
Asparagine*	58.61 ± 8.64	69.62 ± 7.74	71.04 ± 7.86	70.01 ± 7.17	68.69 ± 7.52	71.91 ± 8.91	70.39 ± 9.07	60.79 ± 7.73	57.65 ± 7.36
Citrulline*	27.33 ± 5.64	26.34 ± 5.02	25.23 ± 4.52	24.49 ± 4.58	24.95 ± 4.25	27.04 ± 4.62	27.44 ± 4.41	28.60 ± 5.12	29.39 ± 5.39
Cystine	105.92 ± 14.56	111.54 ± 21.16	112.37 ± 17.26	109.31 ± 10.28	107.98 ± 14.50	113.21 ± 19.56	111.27 ± 22.21	105.41 ± 16.24	101.71 ± 14.19
Ethanolamine	10.46 ± 3.11	9.30 ± 2.83	8.97 ± 2.65	9.00 ± 2.42	8.89 ± 2.88	8.90 ± 2.60	9.35 ± 2.41	9.64 ± 2.72	9.57 ± 2.52
Glutamate*	33.27 ± 20.76	47.14 ± 20.83	44.14 ± 17.27	42.92 ± 20.24	42.44 ± 26.19	44.07 ± 22.85	45.83 ± 22.46	37.67 ± 23.14	28.35 ± 15.50
Glutamine	589.80 ± 74.31	603.87 ± 105.54	620.47 ± 109.03	607.43 ± 48.79	586.07 ± 79.73	599.07 ± 73.80	583.87 ± 69.12	602.67 ± 58.75	610.67 ± 57.87
Glycine	182.53 ± 35.98	179.60 ± 37.36	180.07 ± 49.47	172.79 ± 30.39	169.13 ± 38.92	170.80 ± 35.94	170.60 ± 28.02	177.13 ± 27.73	185.07 ± 27.51
Histidine	89.53 ± 8.98	90.20 ± 6.79	97.56 ± 7.21	100.10 ± 7.79	98.47 ± 8.53	96.41 ± 8.41	97.05 ± 9.25	86.70 ± 8.16	83.61 ± 7.14
Hydroxyproline	6.05 ± 1.14	6.15 ± 1.24	6.47 ± 1.70	6.03 ± 0.95	5.92 ± 1.17	6.31 ± 0.85	6.03 ± 1.01	6.10 ± 0.97	6.22 ± 0.92
Isoleucine*	80.09 ± 11.17	109.05 ± 13.15	118.77 ± 17.49	123.56 ± 15.49	120.27 ± 19.78	119.39 ± 19.03	113.66 ± 18.21	84.08 ± 18.48	73.29 ± 9.89
Leucine*	156.53 ± 18.49	208.93 ± 25.06	221.87 ± 30.75	231.21 ± 27.71	223.33 ± 36.46	221.73 ± 34.72	206.87 ± 30.78	149.87 ± 29.44	136.13 ± 22.42
Lysine*	168.80 ± 33.76	198.67 ± 35.94	206.53 ± 35.89	205.71 ± 29.85	202.93 ± 37.44	206.53 ± 27.77	197.40 ± 31.43	160.13 ± 35.18	141.80 ± 19.61
Ornithine*	46.79 ± 12.63	51.87 ± 11.82	54.97 ± 14.58	51.71 ± 8.93	48.57 ± 12.27	49.22 ± 9.67	44.51 ± 10.19	42.85 ± 10.42	36.97 ± 8.04
P-ethanolamine*	3.19 ± 0.74	3.03 ± 0.63	2.62 ± 0.50	2.70 ± 0.80	2.77 ± 0.61	2.81 ± 0.67	3.05 ± 0.54	3.43 ± 0.82	3.71 ± 1.03
Phenylalanine*	61.61 ± 7.05	73.04 ± 8.95	75.35 ± 7.60	77.36 ± 6.26	76.29 ± 6.54	79.63 ± 7.74	78.54 ± 6.96	66.58 ± 6.67	61.90 ± 6.63
Proline*	219.87 ± 38.30	268.40 ± 46.80	296.33 ± 37.26	300.64 ± 30.27	308.00 ± 44.01	324.93 ± 37.63	322.80 ± 54.11	262.60 ± 57.62	218.13 ± 44.72
Sarcosine	4.36 ± 0.55	4.96 ± 0.81	4.84 ± 0.73	5.00 ± 0.65	4.83 ± 0.65	4.97 ± 0.65	4.99 ± 0.49	4.64 ± 0.47	4.57 ± 0.62
Serine*	95.46 ± 16.74	111.21 ± 15.60	115.43 ± 14.47	109.34 ± 12.36	112.15 ± 12.03	106.71 ± 9.82	106.63 ± 11.53	97.85 ± 15.47	94.95 ± 14.95
Taurine	57.27 ± 6.18	57.82 ± 6.90	54.37 ± 4.75	55.70 ± 7.44	55.40 ± 6.00	53.03 ± 4.91	54.21 ± 4.70	57.41 ± 6.54	57.23 ± 4.86
Threonine*	96.23 ± 22.07	108.78 ± 26.19	118.25 ± 31.36	113.83 ± 20.91	114.11 ± 24.24	118.23 ± 20.72	114.83 ± 21.32	102.73 ± 24.53	97.78 ± 19.37
Tryptophan*	51.01 ± 9.30	56.49 ± 8.71	57.79 ± 6.95	59.21 ± 5.89	60.69 ± 7.19	60.92 ± 5.16	61.04 ± 6.57	57.14 ± 6.90	51.19 ± 7.23
Tyrosine*	58.43 ± 9.81	74.42 ± 10.75	78.89 ± 14.79	83.21 ± 10.15	82.13 ± 13.72	87.37 ± 11.67	85.95 ± 12.55	71.65 ± 11.99	65.13 ± 9.79
Valine*	317.67 ± 25.45	366.93 ± 28.55	372.47 ± 39.80	386.07 ± 30.71	381.20 ± 36.74	381.33 ± 42.51	370.93 ± 44.64	311.33 ± 40.06	280.40 ± 26.65
1-M-Histidine	4.70 ± 1.48	5.43 ± 1.60	6.41 ± 4.13	5.10 ± 1.52	4.81 ± 1.87	5.09 ± 1.51	5.09 ± 2.19	4.63 ± 2.18	4.00 ± 1.60
3-M-Histidine	5.22 ± 1.18	5.23 ± 1.10	5.13 ± 1.10	5.19 ± 1.06	5.23 ± 1.59	5.29 ± 1.18	4.96 ± 0.99	5.03 ± 1.17	5.14 ± 1.22
Sum*	3039.32 ± 236.63	3343.88 ± 286.35	3461.84 ± 278.87	3489.95 ± 182.02	3460.07 ± 230.35	3538.65 ± 188.16	3486.09 ± 204.55	3129.60 ± 271.60	2954.14 ± 172.58

Data are presented as mean as mean ± SD. **P-value* < 0.05 obtained by repeated-measurement ANOVA for time-effects and adjusted with Benjamini-Hochberg correction.

Suppl. Table 17: Plasma amino acids CET (HuMet-study)

Volunteers (n=15) underwent a cycling ergometer test (CET) for 30 min at the individual anaerobic threshold and amino acids were determined via LC-MS/MS.

	Time-point						
	33	34	35	36	37	38	39
Amino acids (µmol/l)	Time (minutes)						
	0	15	30	45	60	90	120
α-aminobutyrate	20.24 ± 5.13	19.73 ± 6.44	18.63 ± 5.59	18.71 ± 5.83	19.72 ± 6.01	20.00 ± 6.55	20.41 ± 6.34
Alanine*	412.07 ± 53.52	494.40 ± 54.89	547.36 ± 57.58	448.47 ± 52.71	413.33 ± 65.28	380.73 ± 70.84	381.40 ± 54.95
Arginine*	74.18 ± 10.41	74.23 ± 11.40	70.29 ± 10.42	65.83 ± 9.57	67.01 ± 10.98	69.03 ± 10.47	68.17 ± 9.95
Asparagine*	64.03 ± 6.81	62.17 ± 5.33	59.81 ± 5.42	57.66 ± 5.37	58.77 ± 4.90	58.89 ± 5.02	59.45 ± 5.51
Citrulline*	31.74 ± 6.70	33.67 ± 5.08	33.08 ± 4.97	32.33 ± 4.77	34.41 ± 4.88	36.57 ± 5.12	35.73 ± 4.27
Cystine	106.05 ± 15.55	99.72 ± 11.68	102.99 ± 21.34	101.44 ± 16.78	108.91 ± 16.36	108.79 ± 16.97	108.17 ± 13.06
Ethanolamine*	9.82 ± 3.23	11.66 ± 2.99	13.54 ± 3.57	11.19 ± 2.88	10.16 ± 2.58	9.89 ± 2.20	9.91 ± 2.49
Glutamate*	36.59 ± 24.69	33.36 ± 10.71	34.13 ± 8.78	61.67 ± 15.48	63.19 ± 26.02	49.94 ± 17.58	36.11 ± 18.17
Glutamine*	640.33 ± 43.19	653.47 ± 45.55	704.79 ± 117.73	668.47 ± 113.36	651.47 ± 77.78	654.93 ± 80.33	644.20 ± 65.68
Glycine*	214.27 ± 27.80	217.33 ± 34.35	214.64 ± 30.73	196.20 ± 26.43	197.20 ± 26.29	199.07 ± 30.45	209.33 ± 27.18
Histidine	85.45 ± 7.70	87.98 ± 8.88	83.41 ± 6.84	83.79 ± 4.70	82.57 ± 6.34	83.75 ± 5.79	82.01 ± 4.62
Hydroxyproline	9.07 ± 2.09	9.03 ± 2.52	8.59 ± 2.33	8.39 ± 2.43	8.59 ± 2.08	8.69 ± 1.84	8.93 ± 1.67
Isoleucine	75.75 ± 12.41	74.84 ± 9.78	73.65 ± 9.66	73.29 ± 11.45	74.29 ± 9.95	73.99 ± 8.69	71.46 ± 9.93
Leucine	148.60 ± 20.02	148.73 ± 19.19	145.57 ± 18.04	143.27 ± 16.64	142.47 ± 18.67	140.80 ± 17.55	137.27 ± 17.88
Lysine*	189.33 ± 21.02	182.20 ± 21.48	171.07 ± 18.43	153.33 ± 16.42	156.00 ± 18.58	154.87 ± 16.52	154.13 ± 16.34
Ornithine*	58.31 ± 8.13	55.51 ± 9.85	50.41 ± 5.43	46.65 ± 7.01	46.77 ± 8.40	48.03 ± 6.34	48.34 ± 6.14
P-ethanolamine*	3.73 ± 1.01	4.13 ± 1.02	4.53 ± 0.96	3.87 ± 0.84	3.40 ± 0.68	3.17 ± 0.66	3.46 ± 0.71
Phenylalanine	57.83 ± 4.92	57.59 ± 5.13	58.16 ± 5.43	56.57 ± 3.67	56.63 ± 4.92	56.65 ± 4.15	56.97 ± 2.88
Proline*	240.80 ± 55.27	231.73 ± 53.27	224.07 ± 47.93	219.47 ± 52.22	217.60 ± 49.04	213.87 ± 45.15	208.40 ± 41.98
Sarcosine	4.78 ± 0.63	4.53 ± 0.81	4.79 ± 0.51	4.74 ± 0.64	4.56 ± 0.76	4.37 ± 0.80	4.47 ± 0.68
Serine*	103.85 ± 22.01	98.89 ± 19.89	93.71 ± 18.46	96.51 ± 18.76	101.01 ± 18.13	98.87 ± 18.43	98.71 ± 17.15
Taurine*	57.28 ± 7.94	58.64 ± 8.86	63.84 ± 11.87	60.43 ± 10.29	55.57 ± 9.41	55.60 ± 5.28	55.31 ± 7.33
Threonine*	123.84 ± 24.02	120.08 ± 24.03	116.50 ± 19.21	111.34 ± 18.22	112.72 ± 19.31	112.40 ± 21.85	116.05 ± 19.45
Tryptophan*	50.42 ± 7.13	47.03 ± 6.34	44.67 ± 6.80	44.61 ± 7.54	48.41 ± 6.76	47.67 ± 6.23	47.15 ± 6.85
Tyrosine*	69.33 ± 10.69	67.50 ± 9.13	67.03 ± 10.18	65.25 ± 8.79	66.14 ± 8.88	64.89 ± 8.20	62.79 ± 8.47
Valine*	281.80 ± 28.43	277.27 ± 30.11	263.00 ± 31.28	264.60 ± 29.85	266.67 ± 29.78	259.47 ± 24.54	252.73 ± 26.21
1-M-Histidine	10.32 ± 2.96	9.95 ± 2.95	9.93 ± 2.33	10.12 ± 2.71	10.32 ± 2.93	10.48 ± 2.95	9.62 ± 2.99
3-M-Histidine	4.76 ± 0.99	4.82 ± 1.18	4.91 ± 0.94	5.08 ± 1.01	5.05 ± 1.07	5.23 ± 1.24	5.14 ± 1.25
Sum*	3184.56 ± 240.24	3240.18 ± 256.58	3287.11 ± 284.63	3113.27 ± 243.10	3082.94 ± 207.86	3030.62 ± 237.56	2995.84 ± 183.05

Data are presented as mean as mean ± SD. **P*-value < 0.05 obtained by repeated-measurement ANOVA for time-effects and adjusted with Benjamini-Hochberg correction.

Suppl. Table 18: Plasma amino acids OLTT (HuMet-study)

Volunteers (n=15) were challenged with an oral lipid loading test (OLTT) and amino acids were determined via LC-MS/MS.

	Time-point										
	40	41	42	43	44	45	46	47	48	49	50
Amino acids	Time (min)										
(µmol/l)	0	30	60	90	120	180	240	300	360	420	480
α-aminobutyrate*	30.37 ± 8.94	30.77 ± 7.78	30.19 ± 7.75	29.66 ± 7.44	27.31 ± 4.65	24.88 ± 4.32	25.28 ± 3.79	27.16 ± 4.74	28.31 ± 3.81	30.53 ± 5.08	30.24 ± 4.80
Alanine*	391.00 ± 95.52	384.60 ± 94.90	423.87 ± 109.49	456.53 ± 111.47	498.80 ± 104.61	438.87 ± 78.99	380.87 ± 62.15	362.67 ± 72.85	355.33 ± 43.41	336.73 ± 31.81	357.63 ± 46.46
Arginine*	80.25 ± 9.92	80.77 ± 10.38	78.67 ± 10.91	81.64 ± 8.39	80.77 ± 9.45	72.18 ± 10.63	67.72 ± 6.88	63.19 ± 7.34	62.63 ± 8.07	65.02 ± 8.45	65.65 ± 7.44
Asparagine*	69.51 ± 6.46	71.97 ± 5.29	70.27 ± 5.23	77.17 ± 7.85	77.37 ± 8.48	67.79 ± 7.24	64.77 ± 5.10	63.88 ± 5.90	64.35 ± 5.36	64.70 ± 5.75	67.23 ± 5.91
Citrulline*	35.79 ± 5.00	33.33 ± 5.82	28.69 ± 5.80	25.13 ± 4.72	25.24 ± 5.15	25.17 ± 4.72	22.84 ± 4.11	22.95 ± 3.49	25.22 ± 4.22	28.63 ± 4.64	29.53 ± 3.74
Cystine	114.66 ± 13.41	121.21 ± 19.06	117.51 ± 16.10	115.07 ± 14.21	109.25 ± 19.71	108.05 ± 10.10	106.03 ± 10.54	111.18 ± 18.62	109.27 ± 15.25	110.54 ± 15.17	103.24 ± 16.55
Ethanolamine	10.71 ± 3.14	9.19 ± 2.44	8.81 ± 2.36	8.18 ± 2.61	9.03 ± 3.01	9.19 ± 2.85	9.83 ± 3.73	9.86 ± 3.21	9.54 ± 2.91	9.95 ± 3.04	9.57 ± 1.73
Glutamate*	42.05 ± 17.28	55.71 ± 21.68	44.73 ± 16.94	55.93 ± 16.05	51.70 ± 26.24	46.19 ± 20.52	44.23 ± 27.36	37.43 ± 13.88	30.09 ± 14.05	33.23 ± 15.59	29.99 ± 14.36
Glutamine	665.27 ± 45.86	641.80 ± 65.81	631.47 ± 93.71	631.93 ± 80.31	629.67 ± 67.47	604.53 ± 69.92	629.40 ± 61.10	631.47 ± 58.03	640.80 ± 57.95	649.87 ± 57.88	657.85 ± 79.12
Glycine	245.40 ± 32.09	232.73 ± 30.03	228.43 ± 26.80	220.00 ± 26.21	220.47 ± 26.26	207.47 ± 28.24	201.07 ± 22.36	201.80 ± 26.65	204.27 ± 29.53	209.13 ± 22.63	215.35 ± 20.53
Histidine*	92.55 ± 7.61	92.37 ± 5.85	88.35 ± 7.56	91.65 ± 6.13	93.93 ± 7.60	86.00 ± 7.65	89.21 ± 5.44	84.80 ± 5.14	81.44 ± 7.60	82.01 ± 7.43	79.16 ± 7.88
Hydroxyproline*	7.74 ± 1.16	7.76 ± 1.06	7.71 ± 1.19	7.44 ± 1.06	7.34 ± 0.96	6.86 ± 1.03	6.65 ± 1.04	6.86 ± 1.38	6.99 ± 1.01	7.04 ± 1.16	6.96 ± 1.18
Isoleucine*	83.44 ± 14.37	90.09 ± 11.84	85.05 ± 10.08	93.11 ± 13.42	94.09 ± 13.04	78.55 ± 11.56	77.67 ± 10.61	76.74 ± 7.59	79.53 ± 6.74	83.27 ± 7.46	86.16 ± 9.25
Leucine*	157.53 ± 22.79	172.47 ± 19.64	163.53 ± 17.37	174.53 ± 24.92	175.00 ± 22.92	147.13 ± 17.40	143.93 ± 14.60	142.20 ± 13.08	145.13 ± 13.84	147.53 ± 13.37	152.75 ± 14.43
Lysine*	185.73 ± 14.10	200.00 ± 16.94	202.27 ± 13.08	216.20 ± 20.38	220.33 ± 17.84	199.87 ± 18.42	182.87 ± 10.78	169.53 ± 14.08	164.00 ± 15.73	158.27 ± 10.80	161.14 ± 11.43
Ornithine*	46.51 ± 5.49	52.71 ± 7.24	50.87 ± 6.45	54.79 ± 5.70	53.58 ± 7.55	54.16 ± 8.93	54.91 ± 5.56	49.79 ± 6.03	47.79 ± 4.70	45.67 ± 4.82	42.58 ± 6.76
P-ethanolamine*	3.71 ± 0.97	3.05 ± 0.76	2.94 ± 0.51	2.72 ± 0.67	2.66 ± 0.76	2.85 ± 0.62	3.18 ± 0.74	3.04 ± 0.86	3.29 ± 0.68	3.29 ± 0.69	3.56 ± 0.76
Phenylalanine*	68.42 ± 6.78	70.33 ± 5.21	71.28 ± 6.52	73.55 ± 6.44	73.88 ± 5.97	65.45 ± 4.51	58.89 ± 3.73	56.59 ± 4.35	56.89 ± 4.27	58.04 ± 4.86	60.89 ± 5.11
Proline*	209.20 ± 38.57	219.93 ± 37.75	227.47 ± 47.71	251.87 ± 49.75	273.73 ± 43.58	242.93 ± 46.60	218.67 ± 33.73	201.40 ± 32.68	193.33 ± 31.58	189.53 ± 31.19	183.71 ± 27.50
Sarcosine*	4.45 ± 0.67	4.79 ± 0.96	4.79 ± 0.78	4.85 ± 0.54	4.79 ± 0.56	4.69 ± 0.63	4.70 ± 0.84	4.26 ± 0.56	4.49 ± 0.61	4.23 ± 0.63	4.18 ± 0.63
Serine*	119.67 ± 20.57	122.31 ± 17.62	117.27 ± 16.65	122.64 ± 12.26	119.12 ± 17.92	109.68 ± 18.58	106.31 ± 18.02	107.49 ± 14.07	107.15 ± 16.11	108.77 ± 15.69	113.29 ± 20.38
Taurine*	56.08 ± 8.35	55.00 ± 8.92	55.21 ± 7.37	53.99 ± 7.92	53.11 ± 7.33	55.36 ± 7.15	57.58 ± 6.68	54.25 ± 8.74	55.01 ± 6.98	55.52 ± 7.59	58.09 ± 7.58
Threonine*	124.15 ± 18.86	126.97 ± 19.84	126.34 ± 18.65	133.73 ± 17.09	131.27 ± 19.05	119.21 ± 17.81	112.23 ± 15.68	110.97 ± 16.96	111.40 ± 17.33	111.80 ± 17.01	108.08 ± 16.35
Tryptophan*	57.20 ± 11.07	62.24 ± 9.26	64.76 ± 9.14	61.05 ± 7.52	60.15 ± 9.08	55.81 ± 7.71	50.96 ± 6.20	48.11 ± 7.41	45.49 ± 5.40	46.75 ± 6.41	52.06 ± 5.59
Tyrosine*	66.25 ± 10.66	68.15 ± 6.91	68.77 ± 9.86	72.61 ± 7.54	75.47 ± 8.81	69.19 ± 8.63	65.41 ± 8.71	61.29 ± 8.05	58.58 ± 7.29	57.47 ± 6.60	57.60 ± 6.75
Valine*	275.47 ± 26.62	288.47 ± 25.28	283.47 ± 23.47	297.47 ± 29.60	301.60 ± 30.95	278.07 ± 25.14	260.33 ± 24.72	260.27 ± 20.07	255.27 ± 20.17	257.80 ± 18.45	258.08 ± 18.20
1-M-Histidine*	6.30 ± 1.83	6.94 ± 2.58	5.73 ± 2.20	6.32 ± 2.08	5.74 ± 1.84	5.29 ± 1.47	5.01 ± 1.56	4.75 ± 1.68	4.33 ± 1.26	4.45 ± 1.57	4.12 ± 1.39
3-M-Histidine	5.23 ± 1.06	5.37 ± 1.03	5.23 ± 0.83	5.05 ± 0.96	5.26 ± 1.02	5.16 ± 0.79	4.93 ± 1.07	5.15 ± 1.08	5.14 ± 0.96	5.05 ± 1.05	4.73 ± 0.96
Sum*	3254.65 ± 242.37	3311.04 ± 208.94	3293.66 ± 244.69	3424.84 ± 242.61	3480.68 ± 250.35	3190.57 ± 232.85	3055.45 ± 138.45	2979.06 ± 166.23	2955.09 ± 166.16	2964.82 ± 97.80	3003.42 ± 147.75

Data are presented as mean as mean ± SD. **P-value* < 0.05 obtained by repeated-measurement ANOVA for time-effects and adjusted with Benjamini-Hochberg correction.

Suppl. Table 19: Urine amino acids (HuMet-study)

Volunteers (n=15) were fasted for 36 hours and challenged with a standard liquid diet (SLD), an oral glucose tolerance test (OGTT), an cycling ergometer test (CET) and an oral lipid tolerance test (OLTT) and amino acids were determined via LC-MS/MS.

	Challenge							
	Fasting	Fasting	Fasting	Fasting	Fasting	Fasting/SLD	SLD	SLD
	Time-point							
	1	3	5	7	9	10	12	18
α-aminoadipate	33.67 ± 18.99	35.21 ± 14.09	35.55 ± 11.13	38.14 ± 11.12	43.23 ± 14.34	34.60 ± 9.51	66.64 ± 33.30	63.85 ± 26.80
α-aminobutyrate	14.22 ± 5.38	16.16 ± 6.10	18.84 ± 6.01	24.52 ± 8.50	32.81 ± 9.72	34.78 ± 9.83	31.42 ± 7.00	23.04 ± 4.34
Alanine	310.95 ± 173.53	267.58 ± 94.67	196.37 ± 74.11	153.23 ± 67.42	148.04 ± 61.57	130.17 ± 62.39	273.75 ± 177.03	321.44 ± 113.12
Arginine	118.63 ± 42.57	78.83 ± 44.58	92.55 ± 47.48	105.90 ± 70.76	133.68 ± 76.43	159.07 ± 98.76	124.58 ± 82.83	90.59 ± 49.06
Argininosuccinate	43.88 ± 20.17	33.94 ± 12.06	34.12 ± 9.63	33.88 ± 15.42	48.11 ± 12.63	48.65 ± 12.32	41.97 ± 21.03	49.39 ± 15.54
Asparagine	168.28 ± 182.11	130.05 ± 87.46	124.97 ± 67.99	115.90 ± 58.28	131.26 ± 47.72	134.13 ± 46.21	155.82 ± 84.52	156.40 ± 52.44
Aspartate	2.39 ± 1.16	5.08 ± 2.44	5.43 ± 1.69	6.02 ± 6.28	4.29 ± 2.11	3.18 ± 1.84	5.84 ± 2.62	7.17 ± 6.09
β-aminobutyrate	134.96 ± 99.68	130.42 ± 101.08	194.52 ± 100.29	367.77 ± 219.06	586.01 ± 357.80	758.73 ± 364.49	398.05 ± 253.08	125.25 ± 57.28
Carnosine	49.76 ± 40.56	13.33 ± 7.05	11.24 ± 4.70	15.35 ± 8.07	21.65 ± 13.08	24.80 ± 12.73	19.26 ± 8.77	19.09 ± 8.40
Citrulline	8.44 ± 8.45	6.50 ± 2.23	6.42 ± 3.44	6.11 ± 2.34	7.28 ± 2.11	6.59 ± 2.11	6.27 ± 3.12	7.81 ± 7.85
Cystationine	36.03 ± 26.23	19.48 ± 6.93	13.39 ± 6.36	12.43 ± 7.21	14.34 ± 8.85	17.45 ± 13.07	21.52 ± 12.59	21.73 ± 14.91
Cystine	76.05 ± 59.08	76.63 ± 50.07	79.03 ± 64.15	75.09 ± 36.77	86.45 ± 34.94	94.18 ± 48.49	87.70 ± 44.35	87.08 ± 39.86
Ethanolamine	641.04 ± 264.12	502.74 ± 138.76	556.93 ± 112.63	664.03 ± 129.57	807.28 ± 175.44	886.51 ± 193.70	698.10 ± 147.37	605.91 ± 88.03
Glutamate	14.24 ± 7.58	15.45 ± 6.05	15.81 ± 5.47	19.51 ± 6.99	22.96 ± 7.52	21.93 ± 9.43	20.62 ± 9.96	19.74 ± 8.02
Glutamine	682.62 ± 355.91	658.22 ± 197.26	611.11 ± 211.49	537.01 ± 167.46	559.47 ± 151.81	476.17 ± 138.94	542.42 ± 223.85	554.15 ± 128.58
Glycine	1354.14 ± 738.12	1157.79 ± 245.66	1063.72 ± 283.55	1036.43 ± 267.99	1032.26 ± 318.14	899.56 ± 286.55	1064.27 ± 418.32	1077.21 ± 340.98
Histidine	1058.36 ± 782.54	860.34 ± 385.54	848.53 ± 499.23	879.09 ± 536.24	955.65 ± 534.67	979.00 ± 544.53	1075.69 ± 668.68	1052.94 ± 485.42
Homocitrulline	55.16 ± 35.45	24.83 ± 6.29	25.64 ± 6.50	27.62 ± 8.30	35.01 ± 14.36	35.39 ± 13.50	40.10 ± 13.38	46.89 ± 14.39
Isoleucine	15.70 ± 6.69	12.29 ± 3.37	13.81 ± 3.67	21.56 ± 5.88	28.43 ± 7.85	30.42 ± 6.69	24.43 ± 5.60	18.12 ± 3.71
Leucine	40.11 ± 19.77	32.90 ± 9.04	36.48 ± 9.81	50.02 ± 14.12	67.86 ± 18.48	76.09 ± 19.24	64.24 ± 14.92	49.77 ± 8.93
Lysine	194.48 ± 211.36	178.20 ± 252.65	161.65 ± 230.47	143.81 ± 184.63	159.82 ± 244.77	137.43 ± 158.07	162.87 ± 138.69	192.49 ± 228.24
Methionine	11.59 ± 9.50	9.01 ± 3.70	6.72 ± 4.09	6.46 ± 3.48	7.88 ± 3.14	8.80 ± 4.85	8.81 ± 5.11	9.08 ± 4.87
Ornithine	20.63 ± 10.23	18.34 ± 8.45	15.97 ± 6.90	20.36 ± 14.23	19.81 ± 7.26	17.39 ± 4.37	21.59 ± 5.26	17.81 ± 4.41
P-ethanolamine	49.10 ± 30.26	10.96 ± 5.30	22.40 ± 11.05	32.14 ± 15.92	37.09 ± 20.30	46.35 ± 26.31	8.88 ± 5.41	15.40 ± 8.98
Phenylalanine	69.99 ± 30.04	60.04 ± 20.26	58.86 ± 16.57	66.65 ± 20.66	73.22 ± 18.72	67.34 ± 18.76	78.44 ± 21.72	81.43 ± 19.61
Proline	6.81 ± 3.15	7.88 ± 2.61	6.76 ± 2.11	8.67 ± 3.29	9.51 ± 1.76	8.60 ± 1.75	10.59 ± 2.82	10.97 ± 3.62
Serine	510.81 ± 232.23	417.19 ± 108.79	395.40 ± 87.84	419.53 ± 74.18	461.28 ± 80.71	481.53 ± 82.01	457.10 ± 93.98	429.93 ± 56.47
Taurine	658.60 ± 399.51	761.65 ± 460.35	704.47 ± 432.93	767.46 ± 525.97	697.42 ± 428.81	473.94 ± 332.79	1524.28 ± 806.31	1170.40 ± 706.32
Threonine	155.49 ± 106.90	142.12 ± 46.78	118.11 ± 37.36	102.40 ± 26.69	97.00 ± 28.26	83.44 ± 21.22	112.74 ± 57.75	117.95 ± 40.19
Tryptophan	90.87 ± 49.02	74.78 ± 26.50	74.70 ± 27.40	87.80 ± 38.05	94.43 ± 39.25	82.31 ± 36.04	80.17 ± 35.01	78.20 ± 26.87
Tyrosine	117.39 ± 64.43	112.41 ± 39.10	98.61 ± 33.65	99.87 ± 38.76	100.41 ± 33.90	85.73 ± 32.90	126.38 ± 56.87	143.69 ± 49.14
Valine	48.41 ± 19.84	44.81 ± 11.11	47.13 ± 9.69	62.20 ± 12.72	80.63 ± 16.60	84.91 ± 15.36	79.74 ± 14.62	66.03 ± 11.71
1-M-Histidine	1936.66 ± 832.53	1138.27 ± 406.80	986.20 ± 286.49	917.79 ± 315.05	979.89 ± 274.00	812.55 ± 250.97	510.41 ± 245.37	413.73 ± 159.79
3-M-Histidine	432.61 ± 148.76	342.97 ± 81.43	349.45 ± 66.73	410.51 ± 79.59	522.75 ± 135.04	558.68 ± 117.11	475.14 ± 120.83	418.00 ± 86.05
Sum	8411.73 ± 2920.06	7734.87 ± 1536.89	6917.44 ± 1577.38	7201.03 ± 1746.89	8076.93 ± 1679.06	7587.99 ± 1312.31	8274.84 ± 2115.31	7481.86 ± 1404.59

Suppl. Table 19 continued:

	Challenge						
	OGTT	OGTT	CET	CET	OLTT	OLTT	OLTT
	Time-point						
	21	29	33	39	40	46	50
α-aminoadipate	35.66 ± 19.73	29.34 ± 6.67	52.33 ± 13.67	33.90 ± 10 .26	37.44 ± 11.76	61.58 ± 18.55	47.00 ± 14.47
α-aminobutyrate	14.07 ± 4.75	13.77 ± 5.36	13.73 ± 5.08	11.71 ± 3.39	19.75 ± 3.32	25.15 ± 5.16	23.34 ± 3.54
Alanine	265.04 ± 122.44	283.81 ± 120.48	346.08 ± 118.38	297 .23 ± 96.55	283.17 ± 122.78	439.73 ± 147.12	175.90 ± 61.30
Arginine	25.51 ± 7.91	18.04 ± 7.32	19.94 ± 6.31	21.00 ± 6.93	137.88 ± 79.65	124.55 ± 83.74	104.96 ± 71.95
Argininosuccinate	41.32 ± 13.66	29.02 ± 13.14	33.62 ± 11.86	55.69 ± 3 1.50	63.32 ± 10.85	56.87 ± 15.79	48.93 ± 19.38
Asparagine	142.00 ± 43.51	126.83 ± 63.35	171.84 ± 79.39	146.37 ± 44.10	188.07 ± 67.47	188.01 ± 74.53	148.02 ± 61. 74
Aspartate	1.72 ± 1.36	3.63 ± 1.95	3.74 ± 1.75	6.16 ± 3.51	3.9 8 ± 2.04	7.28 ± 1.94	5.68 ± 2.13
β-aminobutyrate	185.15 ± 179.74	118.77 ± 73.54	104.93 ± 55.48	101.3 0 ± 65.54	227.01 ± 193.27	191.58 ± 151.22	240.29 ± 121.46
Carnosine	41.54 ± 36.73	12.15 ± 5.37	15.87 ± 9.86	18.80 ± 9.0 9	23.34 ± 10.58	19.71 ± 8.86	16.29 ± 8.02
Citrulline	6.57 ± 3.48	4.54 ± 2.25	6.16 ± 2.64	5.20 ± 1.68	7.9 6 ± 1.93	7.03 ± 2.06	5.74 ± 2.04
Cystationine	44.44 ± 26.97	20.00 ± 9.70	26.20 ± 11.80	16.66 ± 10 .47	23.12 ± 16.47	22.81 ± 14.87	13.53 ± 8.13
Cystine	135.56 ± 84.82	141.65 ± 101.06	140.34 ± 111.74	121. 95 ± 66.41	104.39 ± 56.21	109.77 ± 60.02	78.60 ± 40 .56
Ethanolamine	585.69 ± 137.08	567.04 ± 218.45	582.60 ± 228.88	603 .33 ± 144.54	773.07 ± 128.83	707.56 ± 164.10	581.42 ± 104.14
Glutamate	16.79 ± 10.68	16.67 ± 8.08	15.82 ± 4.62	21.50 ± 8.9 7	26.92 ± 12.63	28.61 ± 9.98	21.66 ± 8.28
Glutamine	565.86 ± 134.37	581.28 ± 215.92	641.35 ± 178.37	511 .22 ± 130.18	694.25 ± 185.62	852.15 ± 181.24	618.59 ± 169.95
Glycine	1154.43 ± 422.70	1178.11 ± 355.29	1197.34 ± 328.97	794.29 ± 270.45	1084.72 ± 260.37	1417.13 ± 360.09	1049.49 ± 227.90
Histidine	989.53 ± 410.98	841.75 ± 371.73	1047.57 ± 459.31	67 9.99 ± 381.45	920.96 ± 491.25	1034.49 ± 493.05	815. 66 ± 450.33
Homocitrulline	55.23 ± 15.18	29.90 ± 11.29	40.73 ± 8.70	35.85 ± 12 .08	48.50 ± 14.97	44.05 ± 12.89	39.18 ± 8.60
Isoleucine	17.32 ± 4.58	12.20 ± 3.94	16.45 ± 4.71	18.19 ± 5.47	21.36 ± 4.29	20.74 ± 3.56	21.28 ± 3.74
Leucine	43.34 ± 12.76	33.63 ± 11.70	46.28 ± 10.29	43.06 ± 1 2.53	50.88 ± 10.53	50.96 ± 7.65	45.14 ± 7.82
Lysine	207.29 ± 252.32	188.23 ± 293.78	243.20 ± 355.51	122 .50 ± 129.61	179.52 ± 235.60	192.21 ± 217.77	133.95 ± 157.90
Methionine	14.23 ± 5.89	9.73 ± 4.90	13.83 ± 2.03	11.70 ± 2.83	9.81 ± 3.92	11.12 ± 5.09	7.75 ± 3.99
Ornithine	20.79 ± 7.96	16.36 ± 9.15	20.11 ± 10.01	15.50 ± 4.7 9	20.05 ± 6.82	18.73 ± 6.56	18.55 ± 5.02
P-ethanolamine	52.05 ± 21.74	10.13 ± 6.04	32.34 ± 26.99	44.33 ± 19 .78	50.06 ± 20.62	5.20 ± 2.81	13.21 ± 11.71
Phenylalanine	70.04 ± 21.39	60.32 ± 20.12	74.75 ± 18.38	66.51 ± 2 0.50	86.74 ± 25.81	93.96 ± 20.17	67.32 ± 16.06
Proline	6.45 ± 4.02	3.76 ± 1.18	7.12 ± 2.99	12.60 ± 8.40	10 .54 ± 4.13	13.20 ± 3.38	9.77 ± 2.96
Serine	454.44 ± 83.06	405.01 ± 145.37	462.30 ± 121.21	380. 57 ± 68.19	528.03 ± 69.44	554.60 ± 90.34	418.72 ± 5 9.36
Taurine	610.82 ± 379.93	770.18 ± 408.98	575.40 ± 304.87	613 .06 ± 382.53	463.72 ± 292.11	771.16 ± 620.88	553.36 ± 484.82
Threonine	139.09 ± 42.66	131.27 ± 42.09	160.31 ± 35.54	113.53 ± 26.40	125.46 ± 32.53	181.74 ± 41.79	102.78 ± 26. 11
Tryptophan	86.78 ± 34.03	76.38 ± 30.58	76.94 ± 23.18	74.90 ± 2 9.57	96.67 ± 34.63	95.91 ± 28.69	77.84 ± 26.76
Tyrosine	110.39 ± 40.08	105.51 ± 41.26	139.03 ± 40.68	113.24 ± 38.94	125.73 ± 48.70	157.92 ± 47.56	110.55 ± 36. 73
Valine	51.82 ± 11.77	44.60 ± 14.11	53.64 ± 14.40	58.51 ± 1 5.17	65.85 ± 10.96	68.93 ± 8.17	61.80 ± 9.60
1-M-Histidine	1918.46 ± 635.85	1178.89 ± 480.95	1021.23 ± 332.90	873.14 ± 310.62	799.24 ± 273.57	539.61 ± 214.77	357 .60 ± 111.96
3-M-Histidine	449.18 ± 84.28	351.06 ± 114.01	379.86 ± 97.47	355.5 0 ± 70.20	483.88 ± 86.21	465.05 ± 73.57	399.46 ± 65 .14
Sum	8412.35 ± 1884.73	7525.49 ± 2052.45	7750.35 ± 1273. 08	6378.38 ± 1574.28	7785.38 ± 1534.87	8641.93 ± 13 73.83	6406.96 ± 1138.66

Data are presented as mean as mean ± SD.

Suppl. Table 20: Plasma acylcarnitine concentrations CET (HuMet-study)

Volunteers (n=15) underwent a cycling ergometer test (CET) for 30 min at the individual anaerobic threshold and amino acids were determined via LC-MS/MS.

	Time-point						
	33	34	35	36	37	38	39
	Time (minutes)						
Acylcarnitine (µmol/l)	**0**	**15**	**30**	**45**	**60**	**90**	**120**
Carnitine (C0)	37.273 ± 6.550	36.552 ± 6.689	34.248 ± 5.820	32.160 ± 5.998	30.918 ± 6.113	29.154 ± 5.952	28.981 ± 6.277
Decanoylcarnitine (C10)	0.181 ± 0.072	0.186 ± 0.075	0.184 ± 0.065	0.173 ± 0.084	0.186 ± 0.087	0.196 ± 0.062	0.232 ± 0.089
Decenoylcarnitine (C10:1)	0.101 ± 0.030	0.110 ± 0.033	0.112 ± 0.031	0.104 ± 0.036	0.103 ± 0.036	0.106 ± 0.025	0.114 ± 0.026
Decadienylcarnitine (C10:2)	0.036 ± 0.007	0.035 ± 0.006	0.036 ± 0.007	0.033 ± 0.008	0.033 ± 0.008	0.034 ± 0.006	0.036 ± 0.006
Dodecanoylcarnitine (C12)	0.056 ± 0.024	0.059 ± 0.022	0.061 ± 0.020	0.061 ± 0.028	0.066 ± 0.033	0.070 ± 0.020	0.078 ± 0.023
Dodecanedioylcarnitine (C12-DC)	0.053 ± 0.008	0.053 ± 0.008	0.053 ± 0.012	0.054 ± 0.014	0.053 ± 0.009	0.054 ± 0.010	0.053 ± 0.010
Dodecenoylcarnitine (C12:1)	0.087 ± 0.026	0.094 ± 0.034	0.088 ± 0.028	0.091 ± 0.037	0.097 ± 0.037	0.099 ± 0.029	0.114 ± 0.028
Tetradecanoylcarnitine (C14)	0.027 ± 0.007	0.030 ± 0.007	0.032 ± 0.009	0.032 ± 0.009	0.033 ± 0.012	0.033 ± 0.009	0.038 ± 0.010
Tetradecenoylcarnitine (C14:1)	0.087 ± 0.031	0.091 ± 0.030	0.094 ± 0.035	0.094 ± 0.039	0.104 ± 0.044	0.103 ± 0.029	0.120 ± 0.037
OH-tetradecenoylcarnitine (C14:1-OH)	0.011 ± 0.003	0.012 ± 0.004	0.012 ± 0.003	0.012 ± 0.004	0.013 ± 0.004	0.013 ± 0.003	0.014 ± 0.004
Tetradecadienylcarnitine (C14:2)	0.020 ± 0.010	0.021 ± 0.010	0.022 ± 0.010	0.021 ± 0.012	0.026 ± 0.014	0.026 ± 0.009	0.031 ± 0.012
OH-tetradecadienylcarnitine (C14:2-OH)	0.010 ± 0.002	0.011 ± 0.002	0.011 ± 0.002	0.011 ± 0.002	0.011 ± 0.003	0.011 ± 0.002	0.012 ± 0.002
Hexadecanoylcarnitine (C16)	0.069 ± 0.016	0.075 ± 0.017	0.077 ± 0.017	0.075 ± 0.018	0.075 ± 0.018	0.077 ± 0.019	0.087 ± 0.022
OH-hexadecanoylcarnitine (C16-OH)	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.002	0.006 ± 0.001
Hexadecenoylcarnitine (C16:1)	0.031 ± 0.008	0.034 ± 0.011	0.035 ± 0.010	0.036 ± 0.013	0.038 ± 0.013	0.038 ± 0.011	0.043 ± 0.011
OH-hexadecenoylcarnitine (C16:1-OH)	0.008 ± 0.001	0.009 ± 0.002	0.008 ± 0.001	0.009 ± 0.002	0.009 ± 0.002	0.009 ± 0.002	0.010 ± 0.002
Hexadecadienylcarnitine (C16:2)	0.005 ± 0.002	0.006 ± 0.003	0.006 ± 0.002	0.006 ± 0.002	0.007 ± 0.003	0.007 ± 0.002	0.008 ± 0.003
OH-hexadecadienylcarnitine (C16:2-OH)	0.011 ± 0.002	0.011 ± 0.002	0.012 ± 0.002	0.012 ± 0.002	0.013 ± 0.002	0.012 ± 0.002	0.012 ± 0.002
Octadecanoylcarnitine (C18)	0.033 ± 0.008	0.035 ± 0.008	0.036 ± 0.008	0.034 ± 0.007	0.035 ± 0.007	0.032 ± 0.008	0.036 ± 0.008
Octadecenoylcarnitine (C18:1)	0.095 ± 0.022	0.102 ± 0.025	0.104 ± 0.026	0.105 ± 0.033	0.112 ± 0.032	0.111 ± 0.026	0.127 ± 0.035
OH-octadecenoylcarnitine (C18:1-OH)	0.009 ± 0.002	0.009 ± 0.002	0.010 ± 0.002	0.009 ± 0.002	0.010 ± 0.002	0.010 ± 0.002	0.011 ± 0.002
Octadecadienylcarnitine (C18:2)	0.035 ± 0.007	0.038 ± 0.008	0.040 ± 0.009	0.039 ± 0.011	0.042 ± 0.010	0.038 ± 0.008	0.045 ± 0.012
Acetylcarnitine (C2)	4.702 ± 0.999	6.537 ± 1.211	9.288 ± 1.692	9.868 ± 1.809	9.836 ± 1.584	9.978 ± 1.740	10.758 ± 1.897
Propionylcarnitine (C3)	0.397 ± 0.093	0.419 ± 0.074	0.530 ± 0.197	0.455 ± 0.122	0.398 ± 0.097	0.355 ± 0.085	0.318 ± 0.075
OH-Butyrylcarnitine (C3-DC, C4-OH)	0.067 ± 0.033	0.066 ± 0.024	0.067 ± 0.024	0.072 ± 0.028	0.074 ± 0.027	0.083 ± 0.032	0.082 ± 0.025
OH-valerylcarnitine (C5-OH, C3-M-DC)	0.038 ± 0.024	0.041 ± 0.032	0.047 ± 0.048	0.038 ± 0.015	0.039 ± 0.021	0.044 ± 0.037	0.038 ± 0.014
OH-propionylcarnitine (C3-OH)	0.033 ± 0.016	0.034 ± 0.015	0.033 ± 0.014	0.032 ± 0.014	0.033 ± 0.015	0.033 ± 0.016	0.035 ± 0.016
Propenoylcarnitine (C3:1)	0.011 ± 0.005	0.011 ± 0.004	0.012 ± 0.005	0.012 ± 0.004	0.011 ± 0.003	0.011 ± 0.004	0.013 ± 0.005
Butyrylcarnitine (C4)	0.196 ± 0.033	0.218 ± 0.032	0.237 ± 0.043	0.228 ± 0.039	0.214 ± 0.037	0.194 ± 0.038	0.184 ± 0.031
Butenylcarnitine (C4:1)	0.020 ± 0.005	0.020 ± 0.006	0.021 ± 0.005	0.021 ± 0.006	0.020 ± 0.006	0.021 ± 0.005	0.021 ± 0.006
Hexanoylcarnitine (C6, C4:1-DC)	0.044 ± 0.009	0.050 ± 0.012	0.051 ± 0.011	0.054 ± 0.013	0.053 ± 0.012	0.052 ± 0.012	0.057 ± 0.012

Suppl. Table 20 continued:

	Time-point						
	33	34	35	36	37	38	39
	Time (minutes)						
Acylcarnitine (µmol/l)	**0**	**15**	**30**	**45**	**60**	**90**	**120**
Valerylcarnitine (C5)	0.156 ± 0.040	0.149 ± 0.028	0.140 ± 0.020	0.136 ± 0.024	0.135 ± 0.024	0.128 ± 0.021	0.131 ± 0.019
Glutarylcarnitine (C5-DC, C6-OH)	0.022 ± 0.012	0.023 ± 0.014	0.023 ± 0.011	0.022 ± 0.008	0.022 ± 0.006	0.022 ± 0.010	0.024 ± 0.012
Methylglutarylcarnitine (C5-M-DC)	0.031 ± 0.012	0.029 ± 0.012	0.032 ± 0.018	0.030 ± 0.010	0.029 ± 0.008	0.032 ± 0.013	0.031 ± 0.010
Tiglylcarnitine (C5:1)	0.026 ± 0.011	0.025 ± 0.006	0.028 ± 0.009	0.027 ± 0.008	0.026 ± 0.007	0.027 ± 0.011	0.027 ± 0.009
Glutaconylcarnitine (C5:1-DC)	0.013 ± 0.005	0.012 ± 0.003	0.014 ± 0.004	0.014 ± 0.004	0.013 ± 0.004	0.014 ± 0.004	0.014 ± 0.005
Hexenoylcarnitine (C6:1)	0.019 ± 0.006	0.019 ± 0.005	0.020 ± 0.006	0.020 ± 0.006	0.019 ± 0.006	0.019 ± 0.005	0.021 ± 0.006
Pimelylcarnitine (C7-DC)	0.026 ± 0.011	0.026 ± 0.008	0.027 ± 0.009	0.026 ± 0.010	0.030 ± 0.012	0.036 ± 0.011	0.041 ± 0.013
Octanoylcarnitine (C8)	0.108 ± 0.035	0.111 ± 0.033	0.113 ± 0.027	0.109 ± 0.037	0.116 ± 0.038	0.118 ± 0.025	0.136 ± 0.047
Octenoylcarnitine (C8:1)	0.092 ± 0.038	0.111 ± 0.049	0.118 ± 0.045	0.108 ± 0.038	0.104 ± 0.036	0.102 ± 0.035	0.103 ± 0.035
Nonaylcarnitine (C9)	0.045 ± 0.020	0.047 ± 0.021	0.049 ± 0.021	0.047 ± 0.020	0.046 ± 0.022	0.045 ± 0.020	0.047 ± 0.022

Data are presented as mean as mean ± SD.

Suppl. Table 21: Plasma acylcarnitine concentrations during fasting (HuMet-study)

Volunteers (n=15) were fasted for 36 hours and amino acids were determined via LC-MS/MS.

	Time-point									
	1	2	3	4	5	6	7	8	9	10
	Time (minutes)									
Acylcarnitine (µmol/l)	**0**	**120**	**240**	**360**	**480**	**600**	**720**	**840**	**960**	**1440**
Carnitine (C0)	39.649 ± 7.180	41.045 ± 6.796	39.869 ± 6.015	38.152 ± 5.467	35.600 ± 5.944	34.621 ± 5.251	33.554 ± 5.944	32.839 ± 6.080	33.356 ± 6.058	33.973 ± 8.237
Decanoylcarnitine (C10)	0.223 ± 0.059	0.291 ± 0.099	0.295 ± 0.092	0.267 ± 0.071	0.291 ± 0.066	0.301 ± 0.114	0.335 ± 0.139	0.257 ± 0.116	0.257 ± 0.087	0.330 ± 0.103
Decenoylcarnitine (C10:1)	0.114 ± 0.038	0.139 ± 0.042	0.142 ± 0.051	0.128 ± 0.022	0.138 ± 0.049	0.138 ± 0.044	0.148 ± 0.049	0.116 ± 0.041	0.121 ± 0.031	0.153 ± 0.056
Decadienylcarnitine (C10:2)	0.032 ± 0.008	0.036 ± 0.007	0.037 ± 0.010	0.033 ± 0.004	0.032 ± 0.007	0.033 ± 0.006	0.035 ± 0.006	0.031 ± 0.006	0.031 ± 0.004	0.035 ± 0.007
Dodecanoylcarnitine (C12)	0.082 ± 0.026	0.096 ± 0.037	0.101 ± 0.033	0.092 ± 0.027	0.107 ± 0.019	0.109 ± 0.032	0.128 ± 0.041	0.115 ± 0.055	0.118 ± 0.044	0.144 ± 0.049
Dodecanedioylcarnitine (C12-DC)	0.054 ± 0.011	0.053 ± 0.013	0.053 ± 0.007	0.053 ± 0.009	0.054 ± 0.011	0.053 ± 0.010	0.055 ± 0.011	0.053 ± 0.008	0.054 ± 0.010	0.050 ± 0.010
Dodecenoylcarnitine (C12:1)	0.100 ± 0.025	0.121 ± 0.026	0.124 ± 0.026	0.121 ± 0.026	0.141 ± 0.017	0.147 ± 0.037	0.165 ± 0.054	0.142 ± 0.052	0.153 ± 0.055	0.194 ± 0.064
Tetradecanoylcarnitine (C14)	0.035 ± 0.010	0.037 ± 0.010	0.040 ± 0.011	0.040 ± 0.008	0.045 ± 0.009	0.045 ± 0.011	0.054 ± 0.016	0.054 ± 0.018	0.059 ± 0.023	0.062 ± 0.022
Tetradecenoylcarnitine (C14:1)	0.091 ± 0.015	0.104 ± 0.018	0.109 ± 0.023	0.113 ± 0.026	0.134 ± 0.023	0.138 ± 0.039	0.164 ± 0.057	0.158 ± 0.055	0.172 ± 0.065	0.209 ± 0.080
OH-tetradecenoylcarnitine (C14:1-OH)	0.012 ± 0.003	0.013 ± 0.002	0.015 ± 0.002	0.015 ± 0.003	0.017 ± 0.002	0.016 ± 0.003	0.017 ± 0.004	0.016 ± 0.004	0.017 ± 0.004	0.019 ± 0.006
Tetradecadienylcarnitine (C14:2)	0.023 ± 0.010	0.028 ± 0.010	0.031 ± 0.015	0.029 ± 0.005	0.037 ± 0.014	0.037 ± 0.013	0.047 ± 0.020	0.040 ± 0.017	0.044 ± 0.018	0.063 ± 0.033
OH-tetradecadienylcarnitine (C14:2-OH)	0.012 ± 0.003	0.013 ± 0.003	0.013 ± 0.003	0.012 ± 0.002	0.013 ± 0.004	0.013 ± 0.003	0.013 ± 0.003	0.014 ± 0.003	0.014 ± 0.004	0.014 ± 0.003
Hexadecanoylcarnitine (C16)	0.095 ± 0.019	0.101 ± 0.020	0.108 ± 0.021	0.115 ± 0.026	0.125 ± 0.028	0.131 ± 0.027	0.139 ± 0.035	0.147 ± 0.035	0.153 ± 0.040	0.141 ± 0.034
OH-hexadecanoylcarnitine (C16-OH)	0.006 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.002	0.007 ± 0.002	0.007 ± 0.002	0.008 ± 0.002	0.009 ± 0.003	0.008 ± 0.003	0.009 ± 0.003
Hexadecenoylcarnitine (C16:1)	0.033 ± 0.007	0.035 ± 0.007	0.039 ± 0.009	0.040 ± 0.008	0.046 ± 0.008	0.048 ± 0.011	0.054 ± 0.015	0.055 ± 0.016	0.059 ± 0.019	0.064 ± 0.021
OH-hexadecenoylcarnitine (C16:1-OH)	0.009 ± 0.002	0.010 ± 0.002	0.010 ± 0.002	0.010 ± 0.002	0.011 ± 0.002	0.012 ± 0.002	0.012 ± 0.003	0.013 ± 0.003	0.014 ± 0.004	0.013 ± 0.004
Hexadecadienylcarnitine (C16:2)	0.006 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.009 ± 0.002	0.009 ± 0.003	0.011 ± 0.005	0.011 ± 0.005	0.013 ± 0.006	0.015 ± 0.008
OH-hexadecadienylcarnitine (C16:2-OH)	0.012 ± 0.002	0.012 ± 0.002	0.014 ± 0.002	0.013 ± 0.002	0.014 ± 0.003	0.013 ± 0.002	0.014 ± 0.002	0.015 ± 0.003	0.015 ± 0.004	0.015 ± 0.003
Octadecanoylcarnitine (C18)	0.038 ± 0.008	0.039 ± 0.007	0.042 ± 0.009	0.043 ± 0.008	0.045 ± 0.010	0.048 ± 0.011	0.050 ± 0.011	0.052 ± 0.012	0.055 ± 0.013	0.051 ± 0.009
Octadecenoylcarnitine (C18:1)	0.088 ± 0.019	0.097 ± 0.020	0.105 ± 0.021	0.118 ± 0.025	0.133 ± 0.034	0.136 ± 0.031	0.148 ± 0.039	0.155 ± 0.035	0.160 ± 0.042	0.159 ± 0.038
OH-octadecenoylcarnitine (C18:1-OH)	0.009 ± 0.003	0.009 ± 0.002	0.010 ± 0.002	0.010 ± 0.002	0.011 ± 0.002	↑ .011 ± 0.003	0.011 ± 0.002	0.013 ± 0.003	0.013 ± 0. 005	0.014 ± 0.004
Octadecadienylcarnitine (C18:2)	0.033 ± 0.010	0.038 ± 0.010	0.038 ± 0.010	0.042 ± 0.010	0.048 ± 0.015	0.047 ± 0.015	0.053 ± 0.018	0.052 ± 0.017	0.056 ± 0.017	0.058 ± 0.022
Acetylcarnitine (C2)	5.445 ± 1.114	5.629 ± 1.134	5.968 ± 1.004	6.967 ± 0.923	8.674 ± 1.443	9.844 ± 1.969	10.882 ± 2.456	12. 105 ± 3.064	13.236 ± 4.248	15.899 ± 5.030
Propionylcarnitine (C3)	0.402 ± 0.104	0.386 ± 0.108	0.354 ± 0.090	0.344 ± 0.095	0.294 ± 0.084	0.283 ± 0.077	0.280 ± 0.093	0.274 ± 0.089	0.280 ± 0.083	0.328 ± 0.118
OH-Butyrylcarnitine (C3-DC, C4-OH)	0.077 ± 0.040	0.074 ± 0.029	0.069 ± 0.025	0.073 ± 0.032	0.083 ± 0.029	0.093 ± 0.030	0.105 ± 0.029	0.116 ± 0.034	0.134 ± 0.046	0.191 ± 0.096
OH-valerylcarnitine (C5-OH, C3-M-DC)	0.039 ± 0.022	0.045 ± 0.028	0.054 ± 0.059	0.044 ± 0.030	0.045 ± 0.039	0.046 ± 0.058	0.048 ± 0.053	0.036 ± 0.014	0.038 ± 0.018	0.044 ± 0.029
OH-propionylcarnitine (C3-OH)	0.031 ± 0.015	0.035 ± 0.018	0.034 ± 0.014	0.034 ± 0.015	0.034 ± 0.018	0.033 ± 0.017	0.033 ± 0.014	0.034 ± 0.017	0.034 ± 0.017	0.030 ± 0.011
Propenoylcarnitine (C3:1)	0.011 ± 0.004	0.011 ± 0.005	0.013 ± 0.004	0.011 ± 0.004	0.012 ± 0.004	0.010 ± 0.005	0.011 ± 0.004	0.011 ± 0.003	0.011 ± 0.005	0.011 ± 0.004
Butyrylcarnitine (C4)	0.195 ± 0.028	0.194 ± 0.028	0.179 ± 0.029	0.173 ± 0.030	0.163 ± 0.029	0.164 ± 0.034	0.167 ± 0.034	0.156 ± 0.036	0.165 ± 0.029	0.199 ± 0.047
Butenylcarnitine (C4:1)	0.021 ± 0.005	0.021 ± 0.005	0.023 ± 0.006	0.021 ± 0.006	0.021 ± 0.006	0.023 ± 0.007	0.022 ± 0.005	0.023 ± 0.007	0.022 ± 0.006	0.027 ± 0.008
Hexanoylcarnitine (C6, C4:1-DC)	0.051 ± 0.011	0.057 ± 0.010	0.058 ± 0.008	0.060 ± 0.008	0.066 ± 0.011	0.065 ± 0.013	0.073 ± 0.018	0.068 ± 0.015	0.071 ± 0.017	0.085 ± 0.028

Suppl. Table 21 continued:

	Time-point									
	1	2	3	4	5	6	7	8	9	10
	Time (minutes)									
Acylcarnitine (µmol/l)	**0**	**120**	**240**	**360**	**480**	**600**	**720**	**840**	**960**	**1440**
Valerylcarnitine (C5)	0.153 ± 0.030	0.157 ± 0.041	0.139 ± 0.037	0.149 ± 0.043	0.143 ± 0.039	0.145 ± 0.035	0.142 ± 0.044	0.141 ± 0.040	0.130 ± 0.026	0.165 ± 0.025
Glutarylcarnitine (C5-DC, C6-OH)	0.023 ± 0.007	0.028 ± 0.021	0.026 ± 0.007	0.027 ± 0.017	0.027 ± 0.012	0.026 ± 0.010	0.028 ± 0.010	0.029 ± 0.012	0.031 ± 0.014	0.031 ± 0.020
Methylglutarylcarnitine (C5-M-DC)	0.032 ± 0.013	0.034 ± 0.014	0.033 ± 0.016	0.030 ± 0.010	0.032 ± 0.014	0.034 ± 0.018	0.036 ± 0.016	0.034 ± 0.009	0.037 ± 0.013	0.037 ± 0.010
Tiglylcarnitine (C5:1)	0.028 ± 0.009	0.030 ± 0.015	0.029 ± 0.011	0.027 ± 0.010	0.028 ± 0.012	0.027 ± 0.009	0.029 ± 0.012	0.027 ± 0.009	0.026 ± 0.008	0.029 ± 0.010
Glutaconylcarnitine (C5:1-DC)	0.014 ± 0.004	0.015 ± 0.005	0.015 ± 0.004	0.016 ± 0.005	0.016 ± 0.004	0.015 ± 0.004	0.016 ± 0.003	0.015 ± 0.002	0.016 ± 0.005	0.017 ± 0.004
Hexenoylcarnitine (C6:1)	0.020 ± 0.006	0.021 ± 0.008	0.021 ± 0.006	0.021 ± 0.008	0.021 ± 0.007	0.020 ± 0.007	0.021 ± 0.007	0.020 ± 0.006	0.021 ± 0.008	0.023 ± 0.009
Pimelylcarnitine (C7-DC)	0.039 ± 0.015	0.049 ± 0.018	0.051 ± 0.018	0.045 ± 0.012	0.049 ± 0.015	0.049 ± 0.012	0.050 ± 0.016	0.043 ± 0.018	0.040 ± 0.016	0.045 ± 0.019
Octanoylcarnitine (C8)	0.138 ± 0.045	0.167 ± 0.052	0.167 ± 0.049	0.154 ± 0.036	0.165 ± 0.040	0.176 ± 0.057	0.186 ± 0.060	0.153 ± 0.063	0.148 ± 0.050	0.185 ± 0.060
Octenoylcarnitine (C8:1)	0.066 ± 0.016	0.070 ± 0.016	0.075 ± 0.020	0.078 ± 0.021	0.069 ± 0.016	0.071 ± 0.017	0.076 ± 0.020	0.070 ± 0.019	0.075 ± 0.019	0.080 ± 0.022
Nonaylcarnitine (C9)	0.058 ± 0.024	0.061 ± 0.024	0.062 ± 0.026	0.058 ± 0.029	0.052 ± 0.022	0.051 ± 0.024	0.049 ± 0.023	0.042 ± 0.017	0.043 ± 0.020	0.046 ± 0.020

Data are presented as mean as mean ± SD.

Suppl. Table 22: Plasma acylcarnitine concentrations OGTT (HuMet-study)

Volunteers (n=15) were challenged with an oral glucose tolerance test (75 g glucose) and amino acids were determined via LC-MS/MS.

	Time-point								
	21	22	23	24	25	26	27	28	29
	Time (minutes)								
Acylcarnitines (µmol/l)	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**180**	**240**
Carnitine (C0)	39.744 ± 9.140	39.707 ± 7.679	40.329 ± 8.312	40.651 ± 7.519	41.038 ± 6.955	41.253 ± 7.434	42.621 ± 6.626	41.029 ± 8.280	39.043 ± 8.834
Decanoylcarnitine (C10)	0.236 ± 0.113	0.233 ± 0.091	0.198 ± 0.063	0.177 ± 0.051	0.164 ± 0.065	0.146 ± 0.054	0.146 ± 0.045	0.165 ± 0.076	0.201 ± 0.066
Decenoylcarnitine (C10:1)	0.116 ± 0.035	0.120 ± 0.036	0.105 ± 0.029	0.097 ± 0.022	0.090 ± 0.026	0.083 ± 0.022	0.078 ± 0.024	0.087 ± 0.031	0.102 ± 0.022
Decadienylcarnitine (C10:2)	0.032 ± 0.006	0.033 ± 0.006	0.031 ± 0.008	0.031 ± 0.004	0.030 ± 0.005	0.029 ± 0.006	0.028 ± 0.005	0.030 ± 0.006	0.031 ± 0.005
Dodecanoylcarnitine (C12)	0.084 ± 0.034	0.084 ± 0.034	0.074 ± 0.026	0.071 ± 0.018	0.063 ± 0.021	0.055 ± 0.020	0.054 ± 0.018	0.058 ± 0.025	0.067 ± 0.021
Dodecanedioylcarnitine (C12-DC)	0.053 ± 0.010	0.053 ± 0.013	0.054 ± 0.011	0.054 ± 0.010	0.055 ± 0.010	0.056 ± 0.010	0.055 ± 0.012	0.054 ± 0.010	0.055 ± 0.010
Dodecenoylcarnitine (C12:1)	0.100 ± 0.030	0.105 ± 0.030	0.093 ± 0.029	0.084 ± 0.019	0.074 ± 0.025	0.074 ± 0.022	0.064 ± 0.013	0.076 ± 0.029	0.092 ± 0.029
Tetradecanoylcarnitine (C14)	0.038 ± 0.012	0.038 ± 0.010	0.038 ± 0.011	0.038 ± 0.010	0.035 ± 0.008	0.031 ± 0.007	0.031 ± 0.008	0.030 ± 0.008	0.033 ± 0.009
Tetradecenoylcarnitine (C14:1)	0.095 ± 0.035	0.097 ± 0.036	0.089 ± 0.031	0.083 ± 0.027	0.078 ± 0.030	0.072 ± 0.023	0.069 ± 0.020	0.076 ± 0.032	0.084 ± 0.030
OH-tetradecenoylcarnitine (C14:1-OH)	0.013 ± 0.004	0.013 ± 0.004	0.013 ± 0.004	0.013 ± 0.004	0.011 ± 0.003	0.011 ± 0.003	0.010 ± 0.002	0.011 ± 0.004	0.011 ± 0.003
Tetradecadienylcarnitine (C14:2)	0.025 ± 0.012	0.025 ± 0.011	0.021 ± 0.010	0.018 ± 0.007	0.016 ± 0.007	0.014 ± 0.006	0.013 ± 0.005	0.015 ± 0.010	0.019 ± 0.008
OH-tetradecadienylcarnitine (C14:2-OH)	0.012 ± 0.003	0.012 ± 0.004	0.012 ± 0.004	0.012 ± 0.003	0.012 ± 0.003	0.011 ± 0.003	0.010 ± 0.003	0.010 ± 0.003	0.011 ± 0.003
Hexadecanoylcarnitine (C16)	0.100 ± 0.028	0.100 ± 0.026	0.099 ± 0.028	0.096 ± 0.023	0.093 ± 0.022	0.089 ± 0.022	0.086 ± 0.021	0.080 ± 0.020	0.083 ± 0.023
OH-hexadecanoylcarnitine (C16-OH)	0.006 ± 0.001	0.007 ± 0.002	0.007 ± 0.003	0.007 ± 0.003	0.006 ± 0.002	0.007 ± 0.002	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.002
Hexadecenoylcarnitine (C16:1)	0.037 ± 0.012	0.036 ± 0.010	0.035 ± 0.011	0.034 ± 0.010	0.032 ± 0.010	0.030 ± 0.008	0.028 ± 0.008	0.029 ± 0.009	0.032 ± 0.010
OH-hexadecenoylcarnitine (C16:1-OH)	0.009 ± 0.002	0.010 ± 0.002	0.010 ± 0.003	0.010 ± 0.003	0.009 ± 0.002	0.009 ± 0.002	0.008 ± 0.002	0.008 ± 0.002	0.009 ± 0.002
Hexadecadienylcarnitine (C16:2)	0.006 ± 0.002	0.007 ± 0.002	0.006 ± 0.003	0.006 ± 0.001	0.005 ± 0.002	0.005 ± 0.002	0.004 ± 0.002	0.005 ± 0.002	0.005 ± 0.002
OH-hexadecadienylcarnitine (C16:2-OH)	0.013 ± 0.003	0.013 ± 0.003	0.013 ± 0.002	0.012 ± 0.003	0.012 ± 0.003	0.011 ± 0.002	0.011 ± 0.002	0.012 ± 0.003	0.012 ± 0.003
Octadecanoylcarnitine (C18)	0.041 ± 0.012	0.041 ± 0.012	0.041 ± 0.011	0.043 ± 0.013	0.040 ± 0.009	0.039 ± 0.009	0.037 ± 0.009	0.037 ± 0.010	0.037 ± 0.009
Octadecenoylcarnitine (C18:1)	0.099 ± 0.029	0.101 ± 0.029	0.094 ± 0.025	0.093 ± 0.026	0.084 ± 0.022	0.075 ± 0.019	0.074 ± 0.020	0.077 ± 0.024	0.083 ± 0.026
OH-octadecenoylcarnitine (C18:1-OH)	0.009 ± 0.002	0.010 ± 0.003	0.009 ± 0.002	0.009 ± 0.002	0.009 ± 0.002	0.009 ± 0.002	0.008 ± 0.002	0.009 ± 0.002	0.009 ± 0.002
Octadecadienylcarnitine (C18:2)	0.037 ± 0.009	0.037 ± 0.010	0.036 ± 0.010	0.037 ± 0.009	0.034 ± 0.009	0.031 ± 0.007	0.031 ± 0.008	0.030 ± 0.008	0.033 ± 0.008
Acetylcarnitine (C2)	5.832 ± 1.527	6.109 ± 1.630	5.507 ± 1.403	5.468 ± 1.408	4.917 ± 1.284	4.030 ± 0.577	3.866 ± 0.505	4.188 ± 1.395	4.830 ± 1.386
Propionylcarnitine (C3)	0.384 ± 0.109	0.401 ± 0.094	0.413 ± 0.105	0.400 ± 0.094	0.396 ± 0.092	0.393 ± 0.100	0.387 ± 0.084	0.346 ± 0.087	0.317 ± 0.094
OH-Butyrylcarnitine (C3-DC, C4-OH)	0.066 ± 0.023	0.079 ± 0.028	0.080 ± 0.035	0.083 ± 0.031	0.075 ± 0.036	0.078 ± 0.035	0.067 ± 0.029	0.068 ± 0.029	0.069 ± 0.036
OH-valerylcarnitine (C5-OH, C3-M-DC)	0.033 ± 0.008	0.039 ± 0.018	0.043 ± 0.021	0.037 ± 0.012	0.048 ± 0.053	0.055 ± 0.076	0.042 ± 0.028	0.038 ± 0.023	0.046 ± 0.043
OH-propionylcarnitine (C3-OH)	0.029 ± 0.009	0.030 ± 0.011	0.030 ± 0.013	0.033 ± 0.012	0.032 ± 0.016	0.036 ± 0.020	0.033 ± 0.012	0.033 ± 0.013	0.035 ± 0.016
Propenoylcarnitine (C3:1)	0.010 ± 0.003	0.011 ± 0.004	0.012 ± 0.005	0.012 ± 0.004	0.012 ± 0.004	0.013 ± 0.006	0.011 ± 0.004	0.011 ± 0.004	0.013 ± 0.006
Butyrylcarnitine (C4)	0.191 ± 0.045	0.199 ± 0.042	0.203 ± 0.047	0.193 ± 0.038	0.194 ± 0.039	0.197 ± 0.045	0.189 ± 0.045	0.174 ± 0.037	0.166 ± 0.033
Butenylcarnitine (C4:1)	0.020 ± 0.006	0.020 ± 0.006	0.024 ± 0.008	0.022 ± 0.006	0.023 ± 0.007	0.024 ± 0.009	0.020 ± 0.007	0.020 ± 0.007	0.022 ± 0.008
Hexanoylcarnitine (C6, C4:1-DC)	0.052 ± 0.012	0.050 ± 0.008	0.047 ± 0.009	0.044 ± 0.008	0.043 ± 0.009	0.039 ± 0.009	0.039 ± 0.006	0.041 ± 0.011	0.046 ± 0.010

Suppl. Table 22 continued:

	Time-point								
	21	22	23	24	25	26	27	28	29
	Time (minutes)								
Acylcarnitines (µmol/l)	**0**	**15**	**30**	**45**	**60**	**90**	**120**	**180**	**240**
Valerylcarnitine (C5)	0.151 ± 0.033	0.148 ± 0.033	0.146 ± 0.022	0.146 ± 0.026	0.154 ± 0.026	0.138 ± 0.029	0.151 ± 0.033	0.126 ± 0.032	0.118 ± 0.037
Glutarylcarnitine (C5-DC, C6-OH)	0.022 ± 0.007	0.024 ± 0.010	0.025 ± 0.006	0.023 ± 0.007	0.024 ± 0.008	0.026 ± 0.008	0.024 ± 0.009	0.021 ± 0.005	0.022 ± 0.007
Methylglutarylcarnitine (C5-M-DC)	0.028 ± 0.005	0.029 ± 0.007	0.035 ± 0.017	0.030 ± 0.007	0.032 ± 0.017	0.036 ± 0.022	0.031 ± 0.010	0.030 ± 0.009	0.034 ± 0.016
Tiglylcarnitine (C5:1)	0.028 ± 0.009	0.028 ± 0.008	0.029 ± 0.008	0.028 ± 0.007	0.028 ± 0.008	0.029 ± 0.011	0.029 ± 0.013	0.027 ± 0.008	0.027 ± 0.008
Glutaconylcarnitine (C5:1-DC)	0.013 ± 0.002	0.013 ± 0.002	0.014 ± 0.005	0.013 ± 0.003	0.014 ± 0.003	0.016 ± 0.006	0.014 ± 0.004	0.012 ± 0.002	0.014 ± 0.005
Hexenoylcarnitine (C6:1)	0.019 ± 0.006	0.022 ± 0.006	0.021 ± 0.007	0.021 ± 0.008	0.020 ± 0.006	0.022 ± 0.007	0.020 ± 0.006	0.020 ± 0.007	0.021 ± 0.009
Pimelylcarnitine (C7-DC)	0.039 ± 0.014	0.041 ± 0.016	0.037 ± 0.016	0.032 ± 0.008	0.031 ± 0.010	0.027 ± 0.008	0.026 ± 0.007	0.027 ± 0.012	0.032 ± 0.013
Octanoylcarnitine (C8)	0.138 ± 0.047	0.134 ± 0.036	0.115 ± 0.023	0.104 ± 0.022	0.101 ± 0.025	0.096 ± 0.031	0.093 ± 0.023	0.106 ± 0.034	0.118 ± 0.026
Octenoylcarnitine (C8:1)	0.082 ± 0.043	0.092 ± 0.045	0.088 ± 0.050	0.086 ± 0.047	0.076 ± 0.035	0.084 ± 0.043	0.078 ± 0.036	0.079 ± 0.040	0.077 ± 0.043
Nonaylcarnitine (C9)	0.055 ± 0.022	0.058 ± 0.024	0.058 ± 0.029	0.059 ± 0.025	0.059 ± 0.025	0.057 ± 0.027	0.057 ± 0.026	0.053 ± 0.025	0.057 ± 0.025

Data are presented as mean as mean ± SD.

Suppl. Table 23: Plasma amino acids of male mice after oral glucose load

Amino acids were analyzed via LC-MS/MS. Mice were fasted for 6 hours before administration of either water (t = 0 min), or 300 µl 20% glucose solution (t = 30 min, 60 min, or 120 min) (n=7-11).

Amino acid (µmol/l)	t=0 min	t=30 min	t=60 min	t=120 min	p-value	p-value adj.
α-aminoadipate	5.48 ± 1.25	4.57 ± 1.41	4.39 ± 1.38	4.90 ± 1.22	0.292	0.360
α-aminobutyrate	7.65 ± 2.58	5.15 ± 1.78	4.86 ± 1.13	5.79 ± 1.72	0.014	0.054
Alanine	422.81 ± 78.06	361.97 ± 140.40	317.60 ± 72.16	316.69 ± 49.75	0.073	0.145
Arginine	75.60 ± 5.36	56.23 ± 23.38	49.21 ± 15.15	45.51 ± 26.21	0.007	0.039[b,c]
Asparagine	55.37 ± 21.65	48.56 ± 14.46	42.60 ± 10.21	40.21 ± 8.07	0.166	0.231
Aspartate	8.42 ± 3.72	4.93 ± 2.20	6.13 ± 3.48	5.08 ± 2.35	0.043	0.125
Citrulline	38.94 ± 7.97	32.90 ± 11.61	29.59 ± 4.03	31.80 ± 6.26	0.116	0.176
Ethanolamine	13.91 ± 4.27	12.65 ± 4.88	9.84 ± 1.61	10.69 ± 1.86	0.110	0.176
Glutamate	46.56 ± 11.27	34.26 ± 7.79	30.04 ± 12.13	35.64 ± 9.27	0.008	0.039[a,b]
Glutamine	570.83 ± 57.86	519.77 ± 129.98	518.73 ± 55.56	605.38 ± 74.81	0,143	0.208
Glycine	260.35 ± 61.05	245.56 ± 49.43	218.33 ± 27.52	228.00 ± 23.32	0,243	0.311
Histidine	70.60 ± 11.12	64.91 ± 19.22	61.39 ± 8.78	69.15 ± 12.78	0.529	0.604
Hydroxyproline	18.08 ± 5.19	16.72 ± 4.09	14.01 ± 2.84	13.71 ± 2.69	0.075	0.145
Isoleucine	114.96 ± 34.76	58.83 ± 21.34	79.94 ± 22.93	88.64 ± 29.01	0.001	0.009[b]
Leucine	176.72 ± 57.89	97.37 ± 35.34	130.76 ± 38.76	156.01 ± 54.81	0.004	0.031[a]
Lysine	232.59 ± 30.71	183.71 ± 63.40	174.74 ± 34.32	191.96 ± 41.54	0.039	0.125
Methionine	39.17 ± 26.35	37.09 ± 30.28	46.51 ± 25.54	41.96 ± 30.91	0.915	0.915
Ornithine	45.55 ± 17.53	40.41 ± 11.89	37.50 ± 10.82	47.41 ± 24.88	0.624	0.689
P-ethanolamine	10.48 ± 3.27	5.93 ± 1.70	5.11 ± 1.66	5.38 ± 1.44	0.000	<0.001[a,b,c]
Phenylalanine	80.18 ± 17.98	62.49 ± 14.59	65.79 ± 15.90	71.31 ± 16.23	0.086	0.154
Proline	89.36 ± 20.64	79.83 ± 32.31	66.46 ± 16.03	64.06 ± 17.10	0.092	0.155
Serine	128.48 ± 27.84	116.98 ± 27.56	108.00 ± 17.05	109.33 ± 14.37	0.239	0.311
Taurine	717.28 ± 166.90	616.21 ± 265.08	500.29 ± 109.69	528.46 ± 77.88	0.065	0.145
Threonine	153.84 ± 32.43	135.33 ± 32.07	119.26 ± 21.71	122.49 ± 26.94	0.066	0.145
Tryptophan	69.65 ± 15.64	65.19 ± 11.07	64.24 ± 10.11	58.70 ± 13.75	0.364	0.432
Tyrosine	82.95 ± 22.47	60.52 ± 20.73	60.13 ± 18.18	61.08 ± 23.61	0.056	0.145
Valine	235.35 ± 58.48	153.77 ± 47.61	171.20 ± 38.94	194.36 ± 64.41	0.009	0.039[b]
1-M-Histidine	5.75 ± 4.97	8.17 ± 9.16	7.07 ± 4.82	8.30 ± 6.54	0.818	0.844
3-M-Histidine	4.94 ± 1.13	4.79 ± 0.87	5.07 ± 1.27	5.36 ± 1.24	0.731	0.779
BCAA	527.03 ± 149.71	309.97 ± 102.72	381.90 ± 99.28	439.01 ± 146.69	0.003	0.031[a]
Sum	3781.86 ± 450.13	3134.80 ± 850.58	2948.79 ± 428.78	3167.35 ± 487.84	0.025	0.090

Data are presented as mean ± SD. *P*-value obtained by one-way ANOVA and adjusted with Benjamini-Hochberg correction. [a]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing t=0 min and t=30 min. [b]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing t=0 min and t=60 min. [c]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing t=0 min and t=120 min.

Suppl. Table 24: Plasma acylcarnitines of male mice after oral glucose load

Acylcarnitines were analyzed via LC-MS/MS. Mice were fasted for 6 hours before administration of either water (t = 0 min), or 300 µl 20% glucose solution (t = 30 min, 60 min, or 120 min) (n=6).

Acylcarnitine (µmol/l)	t = 0 min	t = 30 min	t = 60 min	t = 120 min	*p*-value	*p*-value adj.
Carnitine (C0)	13.65 ± 3.13	18.48 ± 3.47	14.69 ± 1.75	12.46 ± 1.70	0.005	0.097
Acetylcarnitine (C2)	25.23 ± 6.89	14.98 ± 2.67	23.00 ± 5.71	23.88 ± 5.04	0.014	0.184
Propenoylcarnitine (C3:1)	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.386	0.643
Propionylcarnitine (C3)	0.85 ± 0.52	0.60 ± 0.32	0.53 ± 0.10	0.47 ± 0.13	0.201	0.473
Butenylcarnitine (C4:1)	0.22 ± 0.10	0.31 ± 0.14	0.25 ± 0.03	0.22 ± 0.02	0.225	0.474
Butyrylcarnitine (C4)	2.23 ± 1.73	1.12 ± 0.80	1.37 ± 0.71	1.35 ± 0.43	0.291	0.558
OH-propionylcarnitine (C3-OH)	0.08 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.00	0.604	0.780
Tiglylcarnitine (C5:1)	0.14 ± 0.08	0.08 ± 0.04	0.12 ± 0.04	0.11 ± 0.03	0.373	0.643
Valerylcarnitine (C5)	0.38 ± 0.14	0.31 ± 0.13	0.31 ± 0.04	0.31 ± 0.08	0.528	0.703
OH-Butyrylcarnitine (C3-DC, C4-OH)	0.40 ± 0.15	0.18 ± 0.07	0.23 ± 0.06	0.29 ± 0.07	0.005	0.097
Hexenoylcarnitine (C6:1)	0.07 ± 0.02	0.06 ± 0.02	0.08 ± 0.02	0.06 ± 0.02	0.512	0.703
Hexanoylcarnitine (C6, C4:1-DC)	0.20 ± 0.07	0.13 ± 0.02	0.17 ± 0.03	0.16 ± 0.02	0.065	0.436
OH-valerylcarnitine (C5-OH, C3-M-DC)	0.16 ± 0.03	0.14 ± 0.03	0.15 ± 0.01	0.14 ± 0.01	0.213	0.473
Glutaconylcarnitine (C5:1-DC)	0.43 ± 0.08	0.44 ± 0.11	0.51 ± 0.11	0.44 ± 0.05	0.431	0.663
Glutarylcarnitine (C5-DC, C6-OH)	0.41 ± 0.08	0.36 ± 0.06	0.41 ± 0.05	0.36 ± 0.02	0.307	0.558
Octanoylcarnitine (C8)	0.19 ± 0.04	0.20 ± 0.04	0.20 ± 0.02	0.18 ± 0.03	0.641	0.789
Nonaylcarnitine (C9)	0.12 ± 0.02	0.11 ± 0.02	0.13 ± 0.03	0.11 ± 0.01	0.199	0.473
Pimelylcarnitine (C7-DC)	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.00	0.870	0.870
Decadienylcarnitine (C10:2)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.686	0.807
Decenoylcarnitine (C10:1)	0.09 ± 0.02	0.08 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.504	0.703
Decanoylcarnitine (C10)	0.29 ± 0.04	0.29 ± 0.04	0.29 ± 0.03	0.27 ± 0.04	0.769	0.831
Dodecenoylcarnitine (C12:1)	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.835	0.869
Dodecanoylcarnitine (C12)	0.12 ± 0.03	0.09 ± 0.01	0.11 ± 0.02	0.11 ± 0.01	0.135	0.436
Tetradecadienylcarnitine (C14:2)	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.071	0.436
Tetradecenoylcarnitine (C14:1)	0.27 ± 0.05	0.23 ± 0.01	0.24 ± 0.02	0.26 ± 0.03	0.106	0.436
Tetradecanoylcarnitine (C14)	0.23 ± 0.15	0.12 ± 0.01	0.15 ± 0.05	0.14 ± 0.03	0.151	0.436
Dodecanedioylcarnitine (C12-DC)	0.45 ± 0.05	0.43 ± 0.03	0.45 ± 0.02	0.42 ± 0.02	0.466	0.691
OH-tetradecadienylcarnitine (C14:2-OH)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.134	0.436
OH-tetradecenoylcarnitine (C14:1-OH)	0.12 ± 0.07	0.06 ± 0.01	0.07 ± 0.02	0.08 ± 0.04	0.150	0.436
Hexadecadienylcarnitine (C16:2)	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.153	0.436
Hexadecenoylcarnitine (C16:1)	0.08 ± 0.02	0.07 ± 0.03	0.06 ± 0.03	0.08 ± 0.02	0.651	0.789
Hexadecanoylcarnitine (C16)	0.19 ± 0.07	0.13 ± 0.02	0.15 ± 0.02	0.14 ± 0.03	0.181	0.473
OH-hexadecadienylcarnitine (C16:2-OH)	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.710	0.812
OH-hexadecenoylcarnitine (C16:1-OH)	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	0.847	0.869
OH-hexadecanoylcarnitine (C16-OH)	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.297	0.558
Octadecadienylcarnitine (C18:2)	0.10 ± 0.05	0.06 ± 0.02	0.06 ± 0.01	0.08 ± 0.03	0.051	0.436
Octadecenoylcarnitine (C18:1)	0.33 ± 0.14	0.20 ± 0.06	0.20 ± 0.04	0.27 ± 0.10	0.084	0.436
Octadecanoylcarnitine (C18)	0.08 ± 0.02	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.418	0.663
OH-octadecenoylcarnitine (C18:1-OH)	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.731	0.813
Methylglutarylcarnitine (C5-M-DC)	0.70 ± 0.11	0.74 ± 0.11	0.85 ± 0.15	0.71 ± 0.10	0.144	0.436

Data are presented as mean ± SD. *P*-value obtained by one-way ANOVA and adjusted with Benjamini-Hochberg correction.

Suppl. Table 25: Muscle amino acids of male mice after oral glucose load

Amino acids were analyzed via LC-MS/MS and normalized by protein concentration. Mice were fasted for 6 hours before administration of either water (t = 0 min), or 300 µl 20% glucose solution (t = 30 min, 60 min, or 120 min) (n=6).

Amino acids (µmol/g protein)	t=0 min	t=30 min	t=60 min	t=120 min	*p*-value	*p*-value adj.
α-aminoadipic acid	0.05 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.190	0.606
α-aminobutyric acid	1.10 ± 0.35	0.83 ± 0.38	0.90 ± 0.16	0.92 ± 0.18	0.401	0.660
Alanine	41.57 ± 5.83	38.72 ± 7.19	36.17 ± 6.23	36.86 ± 5.55	0.458	0.668
Anserine	77.59 ± 12.63	65.90 ± 15.44	79.53 ± 11.02	69.78 ± 7.16	0.191	0.606
Arginine	4.57 ± 2.11	2.98 ± 1.02	4.33 ± 2.14	3.28 ± 0.98	0.298	0.660
Asparagine	2.71 ± 0.58	2.66 ± 0.41	2.24 ± 0.53	2.30 ± 0.62	0.351	0.660
Aspartate	2.08 ± 0.55	1.95 ± 0.71	2.22 ± 0.39	2.44 ± 0.82	0.584	0.730
Carnosine	52.72 ± 8.78	45.45 ± 8.43	57.08 ± 8.51	48.75 ± 6.13	0.103	0.606
Citrulline	1.26 ± 0.18	1.31 ± 0.14	1.22 ± 0.26	1.23 ± 0.46	0.949	0.977
Ethanolamine	0.91 ± 0.15	0.84 ± 0.17	0.69 ± 0.18	0.77 ± 0.21	0.181	0.606
Glutamate	9.51 ± 2.50	9.56 ± 2.21	9.85 ± 1.25	9.32 ± 2.35	0.979	0.979
Glutamine	26.71 ± 2.73	25.84 ± 2.16	23.17 ± 4.49	24.30 ± 6.65	0.519	0.699
Glycine	40.85 ± 5.72	39.08 ± 3.77	41.13 ± 8.18	38.12 ± 8.18	0.843	0.894
Histidine	2.94 ± 0.42	2.79 ± 0.36	2.75 ± 0.55	2.70 ± 0.55	0.842	0.894
Hydroxyproline	4.49 ± 1.32	3.47 ± 1.43	4.89 ± 2.38	3.67 ± 1.27	0.421	0.660
Isoleucine	1.52 ± 0.32	1.29 ± 0.44	1.74 ± 0.65	1.85 ± 0.20	0.150	0.606
Leucine	2.48 ± 0.39	2.27 ± 0.77	2.73 ± 0.74	2.92 ± 0.63	0.349	0.660
Lysine	12.92 ± 6.35	8.36 ± 2.32	12.69 ± 5.90	10.06 ± 3.11	0.310	0.660
Methionine	1.38 ± 0.66	1.95 ± 0.48	2.84 ± 0.64	1.47 ± 0.19	0.000	0.013[a,b,c]
Ornithine	0.35 ± 0.11	0.33 ± 0.09	0.51 ± 0.21	0.51 ± 0.22	0.134	0.606
P-ethanolamine	1.35 ± 0.18	1.32 ± 0.19	1.26 ± 0.25	1.21 ± 0.09	0.573	0.730
Phenylalanine	1.67 ± 0.31	1.38 ± 0.31	1.87 ± 0.44	1.73 ± 0.24	0.101	0.606
Proline	6.67 ± 1.80	4.81 ± 1.29	6.48 ± 1.48	5.66 ± 0.74	0.111	0.606
Sarcosine	0.13 ± 0.04	0.11 ± 0.05	0.16 ± 0.07	0.11 ± 0.03	0.378	0.660
Serine	6.74 ± 1.17	6.51 ± 0.72	6.06 ± 1.22	6.38 ± 1.87	0.838	0.894
Taurine	898.27 ± 75.83	879.76 ± 99.12	900.57 ± 65.28	851.41 ± 48.93	0.649	0.783
Threonine	5.72 ± 0.58	6.14 ± 1.08	5.84 ± 1.31	5.47 ± 0.83	0.698	0.815
Tryptophan	0.39 ± 0.07	0.35 ± 0.09	0.46 ± 0.12	0.39 ± 0.11	0.247	0.660
Tyrosine	2.06 ± 0.44	1.61 ± 0.38	2.47 ± 0.90	2.00 ± 0.40	0.113	0.606
Valine	3.90 ± 0.56	3.46 ± 0.87	4.18 ± 0.83	4.19 ± 0.73	0.323	0.660
1-M-Histidine	0.09 ± 0.01	0.08 ± 0.03	0.08 ± 0.02	0.08 ± 0.01	0.495	0.693
3-M-Histidine	0.13 ± 0.03	0.13 ± 0.03	0.14 ± 0.02	0.12 ± 0.02	0.402	0.660
BCAA	7.89 ± 1.18	7.02 ± 1.89	8.66 ± 2.18	8.97 ± 1.55	0.244	0.660
Sum	1214.84 ± 95.35	1161.27 ± 125.86	1216.28 ± 82.34	1140.05 ± 74.19	0.434	0.660

Data are presented as mean ± SD. *P*-value obtained by one-way ANOVA and adjusted with Benjamini-Hochberg correction. [a]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing t=0 min and t=60 min. [b]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing t=30 min and t=60 min. [c]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing t=60 min and t=120 min.

Suppl. Table 26: Liver amino acids of male mice after oral glucose load

Amino acids were analyzed via LC-MS/MS. Mice were fasted for 6 hours before administration of either water (t = 0 min), or 300 µl 20% glucose solution (t = 30 min, 60 min, or 120 min) (n=6).

Amino acids (µmol/g protein)	t=0 min	t=30 min	t=60 min	t=120 min	*p*-value	*p*-value adj.
α-aminoadipic acid	0.76 ± 0.55	0.47 ± 0.24	0.74 ± 0.84	1.85 ± 2.93	0.445	0.700
α-aminobutyric acid	1.69 ± 0.47	1.51 ± 0.33	1.45 ± 0.45	1.94 ± 0.87	0.462	0.700
Alanine	45.63 ± 11.05	57.15 ± 18.73	62.10 ± 34.11	51.67 ± 14.87	0.592	0.775
Asparagine	2.92 ± 0.88	2.24 ± 0.41	3.17 ± 0.46	2.77 ± 0.75	0.126	0.606
Aspartate	5.93 ± 1.41	6.03 ± 1.96	6.36 ± 1.64	6.14 ± 1.50	0.973	0.995
β-Alanine	2.81 ± 0.56	2.92 ± 1.73	2.42 ± 0.69	2.46 ± 0.44	0.775	0.869
β-aminoisobutyrate	0.35 ± 0.12	0.28 ± 0.14	0.24 ± 0.06	0.30 ± 0.13	0.435	0.700
Citrulline	1.11 ± 0.50	1.04 ± 0.48	1.06 ± 0.62	1.10 ± 0.64	0.995	0.995
Cystathionine	0.42 ± 0.25	0.27 ± 0.16	0.46 ± 0.21	0.36 ± 0.26	0.507	0.727
Ethanolamine	0.72 ± 0.29	0.48 ± 0.13	0.45 ± 0.07	0.42 ± 0.09	0.021	0.606
Glutamate	15.31 ± 3.71	16.13 ± 5.65	15.43 ± 2.93	17.18 ± 4.79	0.875	0.931
Glutamine	43.58 ± 11.92	40.25 ± 8.68	53.85 ± 10.61	48.82 ± 10.81	0.160	0.606
Glycine	37.18 ± 9.19	34.60 ± 4.15	40.19 ± 8.34	36.48 ± 7.18	0.634	0.775
Histidine	6.98 ± 1.65	7.21 ± 1.60	8.50 ± 2.16	6.91 ± 1.77	0.402	0.700
Hydroxyproline	0.47 ± 0.14	0.47 ± 0.17	0.43 ± 0.10	0.40 ± 0.16	0.790	0.869
Isoleucine	4.49 ± 1.36	2.96 ± 0.84	4.31 ± 0.74	3.96 ± 1.41	0.124	0.606
Leucine	7.60 ± 2.26	5.64 ± 1.27	8.36 ± 2.01	7.20 ± 2.45	0.165	0.606
Lysine	9.51 ± 2.29	8.70 ± 3.95	11.36 ± 4.21	9.04 ± 2.94	0.553	0.761
Methionine	1.48 ± 0.25	1.05 ± 0.41	1.44 ± 0.25	1.26 ± 0.45	0.164	0.606
Ornithine	3.87 ± 1.66	2.40 ± 0.67	3.63 ± 0.95	3.25 ± 1.61	0.250	0.640
P-ethanolamine	6.21 ± 1.27	5.93 ± 1.09	5.72 ± 0.58	5.48 ± 0.89	0.625	0.775
Phenylalanine	2.55 ± 0.80	2.10 ± 0.50	2.91 ± 0.60	2.47 ± 0.81	0.272	0.640
Proline	6.70 ± 1.67	5.19 ± 1.79	6.62 ± 1.48	6.03 ± 2.22	0.466	0.700
Sarcosine	2.02 ± 0.62	0.98 ± 0.24	1.69 ± 0.82	1.58 ± 0.71	0.069	0.606
Serine	6.13 ± 5.01	4.53 ± 1.71	6.26 ± 1.84	5.18 ± 1.79	0.710	0.837
Taurine	360.22 ± 78.57	290.78 ± 65.72	357.45 ± 93.86	357.36 ± 72.08	0.365	0.700
Threonine	5.76 ± 2.38	4.76 ± 1.34	6.52 ± 1.02	5.40 ± 1.93	0.393	0.700
Tryptophan	0.72 ± 0.24	0.55 ± 0.14	0.77 ± 0.12	0.64 ± 0.21	0.242	0.640
Tyrosine	3.33 ± 0.91	2.60 ± 0.54	3.38 ± 0.74	2.97 ± 0.74	0.263	0.640
Valine	8.37 ± 2.76	5.79 ± 1.08	8.45 ± 1.76	7.71 ± 2.79	0.165	0.606
BCAA	20.45 ± 6.24	14.39 ± 3.11	21.12 ± 4.06	18.87 ± 6.59	0.142	0.606
Sum	594.83 ± 101.28	515.01 ± 76.19	625.73 ± 161.32	598.33 ± 98.56	0.391	0.700

Data are presented as mean ± SD. *P*-value obtained by one-way ANOVA and adjusted with Benjamini-Hochberg correction.

Suppl. Table 27: Plasma metabolites of male mice after fasting

Metabolite concentration was either determined via LC-MS/MS or via ^{1}H-NMR. Mice were either fasted for 6 hours (control), 12 hours or 24 hours (n=7-8).

Metabolite (µmol/l)	Control	12 h Fasting	24 h Fasting	*p*-value	*p*-value adj
α-aminoadipic acid	13.62 ± 3.69	28.46 ± 12.81	21.57 ± 5.44	0.008	0.019 [a]
α-aminobutyric acid	8.56 ± 2.15	29.93 ± 5.75	35.11 ± 11.31	<0.001	<0.001 [a,b]
Alanine	771.75 ± 132.28	521.25 ± 123.70	554.29 ± 179.56	0.005	0.014 [a,b]
Arginine	125.04 ± 22.12	113.04 ± 20.75	93.83 ± 13.18	0.018	0.037 [b]
Asparagine	105.25 ± 28.39	95.56 ± 30.17	92.09 ± 15.22	0.596	0.662
Aspartate	9.94 ± 3.52	6.94 ± 2.63	10.16 ± 7.58	0.369	0.461
Citrulline	69.99 ± 24.01	57.45 ± 16.63	51.21 ± 8.35	0.140	0.193
Cystine	98.14 ± 34.02	79.70 ± 22.05	118.04 ± 30.96	0.063	0.101
Glutamate	47.99 ± 9.40	43.66 ± 14.71	41.64 ± 9.03	0.553	0.632
Glutamine	1101.75 ± 106.61	938.50 ± 95.65	1054.43 ± 142.11	0.029	0.051
Glycine	427.88 ± 60.44	420.00 ± 57.07	418.86 ± 72.37	0.954	0.979
Histidine	71.79 ± 13.91	82.24 ± 9.67	90.34 ± 13.36	0.029	0.051
Hydroxyproline	24.88 ± 4.76	11.61 ± 3.93	16.96 ± 2.69	<0.001	<0.001 [a,b,c]
Isoleucine	108.21 ± 19.06	146.62 ± 30.57	153.71 ± 21.60	0.003	0.010 [a,b]
Leucine	174.88 ± 22.97	251.00 ± 44.94	272.71 ± 45.40	<0.001	<0.001 [a,b]
Lysine	345.00 ± 62.74	321.25 ± 36.53	291.71 ± 36.69	0.120	0.172
Methionine	24.69 ± 8.63	32.65 ± 9.88	23.24 ± 7.66	0.103	0.153
Ornithine	51.66 ± 12.21	68.22 ± 16.26	64.29 ± 38.99	0.389	0.469
P-ethanolamine	11.14 ± 2.03	11.60 ± 3.07	11.09 ± 1.36	0.889	0.936
Phenylalanine	87.71 ± 9.67	107.08 ± 13.48	104.71 ± 11.51	0.007	0.017 [a,b]
Proline	99.95 ± 21.69	90.94 ± 21.34	110.41 ± 19.34	0.222	0.296
Serine	172.75 ± 31.31	137.25 ± 21.43	163.29 ± 87.32	0.399	0.469
Taurine	759.88 ± 71.04	1084.62 ± 150.54	803.86 ± 107.16	<0.001	<0.001 [a,c]
Threonine	171.75 ± 22.69	174.25 ± 34.27	223.00 ± 36.83	0.008	<0.019 [b,c]
Tryptophan	71.99 ± 9.31	78.50 ± 16.79	81.14 ± 9.62	0.358	0.461
Tyrosine	101.29 ± 19.67	89.00 ± 22.80	74.81 ± 15.58	0.055	0.092
Valine	228.50 ± 31.38	300.75 ± 47.13	299.71 ± 42.42	0.003	0.009 [a,b]
1-M-Histidine	4.26 ± 1.43	3.73 ± 1.17	5.86 ± 1.41	0.017	0.035 [c]
3-M-Histidine	7.63 ± 2.24	12.39 ± 2.65	19.14 ± 2.85	<0.001	<0.001 [a,b,c]
Sum AA	5297.83 ± 555.54	5338.20 ± 413.56	5301.24 ± 765.60	0.989	0.989
Acetate	241.65 ± 26.71	302.76 ± 43.67	283.85 ± 23.67	0.005	0.014 [a]
Acetoacetate	55.21 ± 4.65	166.11 ± 42.14	207.82 ± 86.29	<0.001	<0.001 [a,b]
Acetone	23.43 ± 6.10	30.22 ± 6.87	29.44 ± 4.52	0.074	0.114
Citrate	320.66 ± 44.29	331.25 ± 36.59	322.50 ± 30.92	0.853	0.922
Creatine	136.11 ± 25.69	277.21 ± 21.09	245.49 ± 16.94	<0.001	0.005 [a,b,c]
Lactate	10142.05 ± 1256.11	7040.26 ± 1149.82	7776.62 ± 1830.08	0.001	0.005 [a,b]
Pyruvate	207.73 ± 31.36	141.59 ± 34.26	138.64 ± 34.04	<0.001	0.003 [a,b]
2-OH-butyrate	71.20 ± 26.05	199.63 ± 50.93	192.30 ± 29.17	<0.001	<0.001 [a,b]
2-Phenyl-propionate	110.58 ± 9.93	92.47 ± 8.01	100.13 ± 15.67	0.023	0.044 [a]
3-OH-butyrate	323.04 ± 95.59	1334.06 ± 210.87	1818.75 ± 455.67	<0.001	<0.001 [a,b,c]

Data are presented as mean ± SD. *P*-value obtained by one-way ANOVA and adjusted with Benjamini-Hochberg correction. [a]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing control and 12 h fasting group. [b]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing control and 24 h fasting group.[c]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing 12 h and 24 h fasting group.

Suppl. Table 28: Liver amino acids of male mice after fasting

Amino acids were analyzed via LC-MS/MS. Mice were either fasted for 6 hours (control), 12 hours or 24 hours (n=8).

Amino acid (µmol/g protein)	Control	12 h Fasting	24 h Fasting	*p*-value	*p*-value adj.
α-aminoadipic acid	2.80 ± 1.48	22.97 ± 18.71	16.53 ± 17.76	0.039	0.092
α-aminobutyric acid	1.75 ± 0.76	5.26 ± 2.59	4.22 ± 1.09	0.001	0.005 [a,b]
Alanine	102.60 ± 17.73	79.03 ± 9.38	61.60 ± 20.30	<0.001	<0.001 [a,b]
Asparagine	4.40 ± 0.53	3.25 ± 0.47	2.95 ± 0.38	<0.001	<0.001 [a,b]
Aspartate	9.70 ± 1.78	9.93 ± 2.29	10.43 ± 3.71	0.859	0.919
β-alanine	2.05 ± 1.03	2.42 ± 1.15	1.78 ± 0.42	0.391	0.505
β-aminoisobutyric acid	0.31 ± 0.08	0.57 ± 0.18	0.53 ± 0.20	0.009	0.027 [a,b]
Citrulline	1.39 ± 0.47	1.72 ± 0.59	1.63 ± 0.64	0.486	0.568
Cystathionine	0.33 ± 0.14	0.24 ± 0.17	0.29 ± 0.13	0.532	0.590
Glutamate	28.42 ± 8.68	32.36 ± 5.71	31.82 ± 6.54	0.495	0.568
Glutamine	128.12 ± 11.62	73.79 ± 15.65	81.59 ± 22.05	<0.001	<0.001 [a,b]
Glycine	62.10 ± 7.98	66.71 ± 6.03	58.54 ± 7.01	0.091	0.140
Histidine	12.89 ± 3.09	11.34 ± 0.89	10.26 ± 1.47	0.055	0.096
Hydroxyproline	0.64 ± 0.20	0.64 ± 0.21	0.50 ± 0.12	0.261	0.351
Isoleucine	5.25 ± 0.81	3.97 ± 0.70	3.34 ± 0.53	<0.001	<0.001 [a,b]
Leucine	10.65 ± 2.26	10.25 ± 4.79	6.88 ± 1.04	0.047	0.096
Lysine	11.44 ± 1.71	10.78 ± 1.22	9.36 ± 1.84	0.051	0.096
Methionine	0.59 ± 0.14	0.56 ± 0.13	0.55 ± 0.16	0.896	0.926
Ornithine	4.68 ± 1.10	4.42 ± 1.27	4.50 ± 2.07	0.946	0.946
P-Ethanolamine	6.53 ± 0.90	7.80 ± 1.52	6.93 ± 0.68	0.081	0.133
Phenylalanine	3.42 ± 0.58	2.46 ± 0.26	2.09 ± 0.23	<0.001	<0.001 [a,b]
Proline	6.80 ± 1.24	6.04 ± 0.76	5.40 ± 1.19	0.055	0.096
Sarcosine	0.88 ± 0.42	0.96 ± 0.38	0.57 ± 0.53	0.205	0.303
Serine	5.76 ± 1.67	4.20 ± 1.30	3.84 ± 0.76	0.017	0.045 [b]
Taurine	217.22 ± 38.96	207.87 ± 84.30	179.82 ± 55.98	0.474	0.568
Threonine	6.66 ± 1.92	5.05 ± 1.56	4.94 ± 0.77	0.056	0.096
Tryptophan	0.90 ± 0.11	0.67 ± 0.11	0.58 ± 0.07	<0.001	<0.001 [a,b]
Tyrosine	3.66 ± 0.39	2.83 ± 0.43	2.33 ± 0.36	<0.001	<0.001 [a,b,c]
Valine	9.51 ± 1.21	8.43 ± 1.30	6.43 ± 1.43	<0.001	0.002 [b,c]
Sum	651.43 ± 39.34	580.53 ± 102.43	520.23 ± 63.81	0.007	0.021 [b]

Data are presented as mean ± SD. *P*-value obtained by one-way ANOVA and adjusted with Benjamini-Hochberg correction. [a]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing control and 12 h fasting group. [b]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing control and 24 h fasting group. [c]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing 12 h and 24 h fasting group.

Suppl. Table 29: Skeletal muscle amino acids of male mice after fasting

Amino acids were analyzed via LC-MS/MS. Mice were either fasted for 6 hours (control), 12 hours or 24 hours (n=8).

Amino acid (µmol/g protein)	Control	12 h Fasting	24 h Fasting	p-value	p-value adj.
α-aminoadipic acid	0.17 ± 0.12	0.32 ± 0.12	0.42 ± 0.30	0.061	0.088
α-aminobutyric acid	0.33 ± 0.05	1.04 ± 0.19	1.45 ± 0.48	<0.001	<0.001 [a,b,c]
Alanine	73.00 ± 13.67	57.07 ± 9.90	61.70 ± 2.56	0.012	0.022 [a]
Anserine	117.26 ± 15.02	120.21 ± 17.30	126.98 ± 32.91	0.695	0.695
Arginine	5.73 ± 0.94	4.31 ± 1.30	4.13 ± 1.11	0.018	0.032 [a,b]
Asparagine	4.67 ± 0.70	4.54 ± 0.51	5.16 ± 0.59	0.127	0.161
Aspartate	2.43 ± 0.59	4.66 ± 2.12	6.52 ± 2.30	0.001	0.003 [b]
Carnosine	80.68 ± 7.87	78.50 ± 7.04	87.07 ± 6.45	0.066	0.090
Citrulline	2.94 ± 0.53	1.93 ± 0.33	1.86 ± 0.22	<0.001	<0.001 [a,b]
Glutamate	14.82 ± 2.25	15.97 ± 5.41	18.41 ± 4.27	0.240	0.294
Glutamine	59.44 ± 4.48	44.49 ± 4.86	46.64 ± 7.15	<0.001	<0.001 [a,b]
Glycine	89.23 ± 11.24	50.57 ± 4.75	55.67 ± 6.59	<0.001	<0.001 [a,b]
Histidine	4.12 ± 0.78	3.97 ± 0.22	4.31 ± 0.70	0.553	0.571
Hydroxyproline	4.99 ± 0.73	3.58 ± 0.60	3.95 ± 0.84	0.003	0.006 [a,b]
Isoleucine	2.31 ± 0.42	3.93 ± 0.96	4.44 ± 0.49	<0.001	<0.001 [a,b]
Leucine	3.56 ± 0.53	6.33 ± 1.28	7.20 ± 0.99	<0.001	<0.001 [a,b]
Lysine	15.83 ± 2.72	11.83 ± 3.14	11.98 ± 3.09	0.022	0.036 [a,b]
Methionine	1.51 ± 0.47	2.02 ± 0.66	1.44 ± 0.44	0.083	0.110
Ornithine	0.62 ± 0.23	0.50 ± 0.17	0.57 ± 0.11	0.379	0.417
P-ethanolamine	2.30 ± 0.27	2.25 ± 0.29	2.55 ± 0.63	0.336	0.396
Phenylalanine	2.22 ± 0.29	3.11 ± 0.33	3.26 ± 0.41	<0.001	<0.001 [a,b]
Proline	7.41 ± 1.07	9.23 ± 1.65	10.76 ± 2.30	0.004	0.008 [b]
Sarcosine	0.17 ± 0.05	0.08 ± 0.02	0.05 ± 0.03	<0.001	<0.001 [a,b]
Serine	11.16 ± 0.89	7.96 ± 0.57	8.39 ± 0.85	<0.001	<0.001 [a,b]
Taurine	460.19 ± 40.02	416.24 ± 27.61	450.45 ± 37.26	0.052	0.082
Threonine	9.35 ± 1.13	8.11 ± 0.70	9.85 ± 0.74	0.002	0.006 [a,c]
Tryptophan	0.49 ± 0.04	0.71 ± 0.17	0.72 ± 0.14	0.003	0.006 [a,b]
Tyrosine	2.95 ± 0.37	2.84 ± 0.61	2.62 ± 0.37	0.370	0.417
Valine	5.97 ± 1.11	7.88 ± 1.50	8.54 ± 1.13	0.002	0.004 [a,b]
1-M-Histidine	0.18 ± 0.10	0.14 ± 0.07	0.18 ± 0.06	0.547	0.571
3-M-Histidine	0.11 ± 0.06	0.18 ± 0.08	0.33 ± 0.07	<0.001	<0.001 [b,c]
Sum	986.13 ± 59.37	874.48 ± 49.76	947.59 ± 76.75	0.006	0.012 [a]

Data are presented as mean ± SD. *P*-value obtained by one-way ANOVA and adjusted with Benjamini-Hochberg correction. [a]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing control and 12 h fasting group. [b]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing control and 24 h fasting group. [c]$p < 0.05$ obtained by post-hoc analysis (Tukey) when comparing 12 h and 24 h fasting group.

Suppl. Table 30: Plasma amino acid concentrations of male mice during twelve weeks dietary intervention (study 1)

Wildtype animals were fed a control (10 energy% of fat, n=12) or high-fat diet (45 energy% of fat, n=12) for 12 weeks and amino acid concentrations were monitored every second week and analyzed via LC-MS/MS.

Amino acid (µmol/l)	Week 0		Week 2		Week 4	
	10 E%	45 E%	10 E%	45 E%	10 E%	45 E%
α-aminoadipate	11.06 ± 1.86	11.32 ± 1.73	11.72 ± 2.69	12.25 ± 3.55	9.41 ± 1.44	9.76 ± 2.27
α-aminobutyrate	8.9 ± 3.47	9.05 ± 2.51	10.0 ± 2.63	7.73 ± 1.31	10.55 ± 2.48	6.94 ± 1.33
Alanine	367.25 ± 79.73	363.17 ± 63.33	357.33 ± 35.04	320.42 ± 56.58	477.08 ± 80.75	459.16 ± 132.04
Arginine	70.88 ± 9.57	73.05 ± 12.72	73.88 ± 8.39	91.22 ± 16.79	78.75 ± 9.83	95.45 ± 10.61
Asparagine	54.34 ± 14.15	55.85 ± 8.11	57.48 ± 9.71	63.6 ± 15.18	68.66 ± 13.37	70.09 ± 21.28
Aspartate	6.32 ± 1.18	6.59 ± 1.56	7.06 ± 1.54	6.77 ± 1.42	6.84 ± 0.88	7.49 ± 1.5
Citrulline	40.54 ± 5.61	41.81 ± 7.17	40.64 ± 5.95	54.98 ± 12.22	38.73 ± 6.98	52.7 ± 6.94
Cystine	70.57 ± 9.32	71.05 ± 9.52	50.29 ± 8.82	62.06 ± 16.07	75.55 ± 10.25	80.70 ± 17.65
Ethanolamine	12.07 ± 1.15	11.48 ± 1.04	12.63 ± 1.26	14.59 ± 2.52	10.62 ± 0.68	11.15 ± 1.16
Glutamate	29.22 ± 5.21	28.77 ± 4.41	30.1 ± 4.87	31.48 ± 5.47	31.34 ± 3.0	29.78 ± 5.35
Glutamine	583.42 ± 49.42	573.5 ± 47.21	557.33 ± 63.0	567.17 ± 94.3	565.92 ± 52.62	603.42 ± 59.43
Glycine	302.42 ± 38.35	309.92 ± 41.85	316.0 ± 43.36	297.67 ± 41.37	301.92 ± 37.53	282.42 ± 43.16
Histidine	56.96 ± 5.09	56.98 ± 9.07	50.68 ± 5.41	57.92 ± 9.52	59.4 ± 8.93	66.38 ± 9.97
Hydroxyproline	18.34 ± 2.74	18.11 ± 2.61	14.84 ± 3.43	16.01 ± 3.2	15.93 ± 3.3	13.88 ± 2.61
Isoleucine	78.01 ± 9.42	80.28 ± 10.41	82.02 ± 16.51	85.45 ± 13.75	95.27 ± 16.91	93.40 ± 20.19
Leucine	117.84 ± 16.11	119.87 ± 18.2	122.74 ± 24.37	135.78 ± 21.65	148.45 ± 26.28	147.12 ± 27.44
Lysine	255.25 ± 43.09	268.75 ± 65.49	262.75 ± 26.54	275.92 ± 56.88	283.5 ± 45.59	306.08 ± 63.0
Methionine	66.26 ± 8.04	71.23 ± 15.13	66.75 ± 14.26	65.10 ± 11.36	61.98 ± 7.98	65.13 ± 12.39
Ornithine	34.18 ± 4.7	38.6 ± 10.67	34.06 ±4.15	43.07 ± 8.31	39.73 ± 9.2	46.08 ± 8.06
P-Ethanolamine	3.98 ± 1.12	3.76 ± 0.58	4.87 ± 0.91	5.19 ± 1.43	4.87 ± 0.99	5.18 ± 0.81
Phenylalanine	54.38 ± 4.85	54.68 ± 7.43	56.93 ± 6.41	62.23 ± 9.31	54.19 ± 6.7	56.11 ± 5.61
Proline	66.77 ± 11.71	69.05 ± 11.79	64.67 ± 12.28	64.26 ± 15.94	68.08 ± 14.67	71.17 ± 17.44
Serine	135.4 ± 24.87	141.57 ± 37.43	132.57 ± 10.03	136.9 ± 22.91	154.40 ± 25.75	162.63 ± 32.71
Taurine	295.25 ± 51.63	313.33 ± 66.93	314.08 ± 45.13	328.5 ± 60.91	350.58 ± 56.9	369.83 ± 44.24
Threonine	155.75 ± 30.36	161.58 ± 28.36	154.83 ± 21.92	165.5 ± 30.77	180.83 ± 40.57	201.08 ± 34.89
Tryptophan	76.96 ± 12.16	74.86 ± 8.79	79.79 ± 6.9	80.68 ± 15.32	77.08 ± 7.54	78.04 ± 12.68
Tyrosine	76.32 ± 11.15	80.43 ± 18.01	82.66 ± 11.58	93.82 ± 15.54	84.17 ± 11.27	96.31 ± 17.68
Valine	183.42 ± 18.85	189.33 ± 25.43	190.42 ± 26.56	201.83 ± 31.74	210.17 ± 32.56	224.0 ± 41.0
1-M-Histidine	4.35 ± 0.45	4.11 ± 0.31	4.22 ± 0.54	4.34 ± 0.6	4.45 ± 0.46	3.97 ± 0.5
3-M-Histidine	6.85 ± 0.73	7.04 ± 1.21	6.58 ± 1.46	7.4 ± 1.06	5.82 ± 0.81	5.81 ± 0.83
Sum	3243.22 ± 352.42	3309.09 ± 470.48	3249.92 ± 282.31	3359.78 ± 487.23	3574.25 ± 420.95	3721.25 ± 470.34

Table 30 continued:

Amino acid (µmol/l)	Week 6		Week 8		Week 10	
	10 E%	45 E%	10 E%	45 E%	10 E%	45 E%
α-aminoadipate	13.93 ± 2.09	12.51 ± 3.06	12.29 ± 3.08	10.60 ± 1.59	12.03 ± 1.97	10.38 ± 1.9
α-aminobutyrate	11.42 ± 4.82	8.88 ± 1.93	10.81 ± 3.76	8.56 ± 1.82	1 1.19 ± 3.39	8.10 ± 2.01
Alanine	441.42 ± 81.42	387.83 ± 45.08	395.50 ± 80.52	402.08 ± 105.75	432.67 ± 119.67	353.42 ± 53.38
Arginine	87.22 ± 11.91	95.43 ± 10.81	79.98 ± 11.49	92.19 ± 1 4.04	83.51 ± 7.87	83.01 ± 7.49
Asparagine	62.82 ± 13.55	67.04 ± 17.78	56.83 ± 11.05	66.82 ± 1 3.76	61.41 ± 13.88	56.43 ± 11.66
Aspartate	9.7 ± 1.76	8.24 ± 1.72	6.57 ± 1.77	7.06 ± 1.36	7.76 ± 2.73	7.04 ± 2.14
Citrulline	45.66 ± 6.87	51.66 ± 5.72	43.08 ± 5.18	52.22 ± 9.30	43.68 ± 4.63	50.91 ± 6.32
Cystine	66.78 ± 8.49	73.98 ± 14.63	83.25 ± 17.84	89.04 ± 15 .00	63.71 ± 13.95	69.52 ± 10.71
Ethanolamine	14.5 ± 1.52	15.73 ± 1.98	12.27 ± 1.06	13.52 ± 2.51	12.83 ± 1.22	13.89 ± 2.62
Glutamate	43.55 ± 6.04	39.93 ± 7.41	31.03 ± 6.5	28.98 ± 2.33	34.44 ± 8.13	32.23 ± 6.69
Glutamine	647.83 ± 64.1	658.58 ± 53.66	647.67 ± 47.07	639.75 ± 72.04	593.17 ± 56.04	602.25 ± 59.59
Glycine	352.83 ± 59.27	314.83 ± 24.19	312.33 ± 34.0	281.0 ± 32.37	294.75 ± 49.3	253.83 ± 29.57
Histidine	69.28 ± 7.65	66.85 ± 5.24	64.71 ± 7.13	62.32 ± 7.74	63.62 ± 3.23	62.36 ± 8.62
Hydroxyproline	16.54 ± 3.59	13.58 ± 2.13	13.83 ± 2.4	11.65 ± 1.67	14.22 ± 1.96	11.33 ± 1.82
Isoleucine	112.42 ± 19.96	109.18 ± 11.98	96.83 ± 16.59	95.78 ± 16.12	104.68 ± 19.88	91.21 ± 19.92
Leucine	176.27 ± 30.74	181.87 ± 19.34	145.7 ± 27.74	150.45 ± 27.10	153.95 ± 25.13	146.95 ± 35.07
Lysine	295.25 ± 41.48	284.58 ± 30.52	279.33 ± 40.94	260.00 ± 31.49	290.42 ± 33.49	230.33 ± 38.66
Methionine	65.68 ± 8.16	62.49 ± 6.64	63.74 ± 8.8	69.87 ± 15.30	67.76 ± 13.82	53.39 ± 7.96
Ornithine	43.75 ± 7.51	46.18 ± 5.94	38.18 ± 8.72	43.47 ± 5.41	38.55 ± 5.36	40.43 ± 5.40
P-Ethanolamine	6.6 ± 1.79	6.97 ± 2.25	4.53 ± 1.23	6.26 ± 2.06	5.81 ± 2.43	5.28 ± 1.81
Phenylalanine	65.65 ± 7.15	66.46 ± 7.16	60.09 ± 8.42	60.75 ± 8.16	60.88 ± 6.27	57.93 ± 10.82
Proline	77.09 ± 11.71	68.74 ± 10.99	69.82 ± 14.22	69.63 ± 1 0.27	75.08 ± 18.64	60.28 ± 8.58
Serine	150.15 ± 24.81	149.32 ± 12.62	144.40 ± 21.89	154.73 ± 21.85	147.40 ± 27.96	131.67 ± 16.27
Taurine	402.08 ± 69.47	375.42 ± 95.02	318.0 ± 50.91	364.5 ± 86.56	383.08 ± 74.17	324.42 ± 59.62
Threonine	190.50 ± 36.3	182.83 ± 23.62	171.17 ± 44.54	178.42 ± 24.54	187.33 ± 41.89	157.96 ± 21.07
Tryptophan	84.08 ± 8.92	82.18 ± 11.29	85.63 ± 10.62	89.04 ± 11 .26	85.74 ± 11.13	80.42 ± 11.47
Tyrosine	96.09 ± 17.75	99.45 ± 12.95	83.27 ± 14.89	95.72 ± 1 4.24	80.31 ± 13.63	86.58 ± 15.21
Valine	247.0 ± 33.27	242.92 ± 19.14	218.33 ± 33.93	220.58 ± 29.87	234.58 ± 27.46	205.83 ± 34.73
1-M-Histidine	5.2 ± 0.66	4.81 ± 0.61	5.15 ± 1.35	4.62 ± 0.81	4.78 ± 0.71	4.69 ± 0.63
1-M-Histidine	7.73 ± 1.2	8.10 ± 1.72	7.74 ± 1.4	7.56 ± 0.67	6.8 ± 1.29	7.44 ± 1.09
Sum	3909.05 ± 462.19	3786.59 ± 264.61	3562.05 ± 370.53	3637.14 ± 369.20	3656.13 ± 410.46	3299.50 ± 330.04

Table 30 continued:

Amino acid (µmol/l)	Week 12		week x fat interaction	
	10 E%	45 E%	*p*-value	*p*-value adj.
α-aminoadipate	14.09 ± 2.69	11.39 ± 1.75*	0.027	0.167
α-aminobutyrate	9.86 ± 3.96	8.34 ± 2.08	0.048	0.173
Alanine	392.92 ± 60.38	392.25 ± 67.39	0.432	0.599
Arginine	76.68 ± 4.97	87.92 ± 12.02*	0.02	0.165
Asparagine	60.37 ± 8.67	64.30 ± 11.14	0.618	0.669
Aspartate	8.55 ± 1.72	9.82 ± 2.69	0.133	0.318
Citrulline	44.05 ± 4.59	59.23 ± 9.36**	0.002	0.032
Cystine	65.33 ± 19.65	72.03 ± 16.17	0.913	0.913
Ethanolamine	12.42 ± 1.23	14.32 ± 1.75*	0.05	0.173
Glutamate	39.41 ± 5.57	39.12 ± 7.18	0.812	0.833
Glutamine	598.08 ± 26.76	676.50 ± 41.21***	0.116	0.318
Glycine	313.5 ± 39.94	294.17 ± 24.47	0.152	0.318
Histidine	60.08 ± 4.11	65.53 ± 7.87	0.03	0.167
Hydroxyproline	15.08 ± 1.92	11.81 ± 1.35**	0.001	0.032
Isoleucine	82.18 ± 13.56	83.08 ± 8.72	0.439	0.599
Leucine	122.95 ± 17.23	123.62 ± 13.01	0.796	0.833
Lysine	256.08 ± 33.88	241.17 ± 48.22	0.021	0.165
Methionine	50.02 ± 5.84	47.30 ± 10.61	0.021	0.165
Ornithine	39.66 ± 3.32	49.33 ± 7.76**	0.342	0.519
P-Ethanolamine	7.38 ± 1.04	8.51 ± 1.65	0.155	0.318
Phenylalanine	52.38 ± 5.62	54.14 ± 4.97	0.551	0.651
Proline	68.15 ± 14.47	69.68 ± 19.56	0.235	0.398
Serine	142.12 ± 20.19	156.57 ± 27.26	0.452	0.599
Taurine	440.42 ± 54.37	485.67 ± 63.44	0.042	0.173
Threonine	172.58 ± 42.83	175.17 ± 40.24	0.151	0.318
Tryptophan	82.25 ± 12.84	92.01 ± 11.67	0.346	0.519
Tyrosine	69.55 ± 10.71	85.88 ± 16.71*	0.587	0.669
Valine	191.75 ± 26.99	199.42 ± 27.48	0.146	0.318
1-M-Histidine	5.45 ± 0.65	5.22 ± 0.55	0.601	0.669
3-M-Histidine	8.24 ± 1.22	7.87 ± 1.18	0.469	0.599
Sum	3501.55 ± 315.63	3691.34 ± 333.58	0.184	0.359

Data are presented as mean ± SD. *P*-value obtained by two-way ANOVA with factors diet and week adjusted with Benjamini-Hochberg correction.
For week 12 additionally between-group comparison. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction. Asterisks indicate statistical significance. * $p<0.05$, ** $p < 0.01$, *** $p < 0.001$.

Suppl. Table 31: Plasma amino acid concentrations of male mice after twelve weeks dietary intervention (study 2)

Wildtype animals were fed a control (11 energy% of fat, n=9) or high-fat diet (60 energy% of fat, n=7) for 12 weeks and amino acid concentrations were analyzed via LC-MS/MS.

Amino acids (µmol/l)	11 E%	60 E%	*p*-value	*p*-value adj.
α-aminoadipate	10.04 ± 1.56	7.30 ± 1.17	0.002	0.018
α-aminobutyrate	8.12 ± 2.16	6.46 ± 0.90	0.079	0.234
Alanine	559.00 ± 85.88	661.43 ± 129.65	0.078	0.243
Arginine	93.22 ± 12.29	87.10 ± 17.16	0.419	0.579
Asparagine	56.41 ± 7.53	77.70 ± 13.47	0.001	0.018
Aspartate	9.61 ± 1.91	7.88 ± 1.57	0.073	0.234
Citrulline	48.41 ± 9.11	61.31 ± 9.49	0.015	0.088
Cystine	15.99 ± 8.49	21.24 ± 11.95	0.32	0.493
Ethanolamine	13.12 ± 2.22	13.50 ± 2.40	0.747	0.803
Glutamate	63.79 ± 14.72	51.57 ± 10.06	0.082	0.234
Glutamine	707.44 ± 58.48	793.86 ± 109.66	0.061	0.234
Glycine	233.56 ± 16.09	218.43 ± 28.15	0.195	0.409
Histidine	74.67 ± 8.02	74.63 ± 6.58	0.992	0.992
Hydroxyproline	16.18 ± 2.22	16.71 ± 2.58	0.661	0.756
Isoleucine	138.00 ± 23.43	130.57 ± 19.39	0.510	0.68
Leucine	222.44 ± 37.79	203.43 ± 32.83	0.309	0.493
Lysine	329.11 ± 39.52	349.00 ± 50.87	0.393	0.579
Methionine	48.60 ± 10.44	53.10 ± 11.12	0.42	0.579
Ornithine	55.20 ± 11.98	82.53 ± 20.07	0.004	0.032
P-Ethanolamine	4.94 ± 2.01	6.15 ± 2.58	0.307	0.493
Phenylalanine	71.97 ± 11.63	69.04 ± 11.53	0.624	0.734
Proline	128.72 ± 34.53	160.27 ± 44.82	0.133	0.313
Sarcosine	1.43 ± 0.49	1.13 ± 0.27	0.167	0.372
Serine	143.22 ± 18.43	182.71 ± 28.71	0.005	0.032
Taurine	310.78 ± 65.43	386.86 ± 96.29	0.080	0.234
Threonine	191.67 ± 36.21	228.71 ± 36.76	0.063	0.234
Tryptophan	74.01 ± 13.13	70.53 ± 9.62	0.566	0.686
Tyrosine	92.47 ± 15.15	97.84 ± 20.70	0.557	0.686
Valine	346.00 ± 56.85	312.86 ± 37.35	0.205	0.409
3-M-Histidine	3.58 ± 0.91	5.80 ± 1.40	0.002	0.018
Sum	4074.51 ± 368.90	4442.52 ± 537.81	0.126	0.313

Data are presented as mean ± SD. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction.

Suppl. Table 32: List of identified metabolites measured in plasma of male mice after 12 weeks of dietary intervention on control or high-fat diet (study 3)

Wildtype animals were fed a control (12 energy% of fat, n=6) or high-fat diet (48 energy% of fat, n=5) for 12 weeks and metabolites were measured by GC-MS.

Metabolite (normalized peak intensity)	12 E%	48 E%	*p*-value
Acetoacetic acid	26.21 ± 11.54	30.31 ± 17.51	0.652
Alanine	4334.65 ± 1603.80	4111.18 ± 898.88	0.789
Alanine CO_2	30.20 ± 8.29	67.96 ± 63.54	0.179
Arginine(-NH_3)	23.68 ± 19.76	28.46 ± 27.64	0.745
Asparagine	364.75 ± 233.17	255.60 ± 74.64	0.345
Asparagine (H_2O)	11.83 ± 8.49	4.81 ± 7.56	0.186
Aspartic acid	63.44 ± 27.36	38.12 ± 17.96	0.111
Cholesterol	6895.07 ± 2204.96	11034.02 ± 1683.64	0.007
Cholesterol (derivate)	55.84 ± 22.96	95.09 ± 26.57	0.027
Citric acid	1793.09 ± 686.45	1829.29 ± 613.95	0.929
Citrulline	25.27 ± 31.86	29.55 ± 19.57	0.800
Creatinine	189.39 ± 60.96	166.85 ± 49.19	0.523
Cysteine	43.74 ± 7.05	50.42 ± 10.30	0.233
Cystine	248.15 ± 57.94	218.07 ± 90.67	0.520
Diethanolamine	8.94 ± 9.50	1.72 ± 2.12	0.133
Diethylenglycol	120.31 ± 92.92	132.14 ± 64.28	0.816
Docosahexaenoic acid	120.69 ± 32.72	123.57 ± 28.71	0.882
Eicosatetraenoic acid	208.79 ± 49.94	255.99 ± 51.99	0.160
Erythronic acid	29.94 ± 10.08	32.35 ± 5.48	0.645
Ethanolaminephosphate	5.87 ± 6.02	9.94 ± 9.99	0.424
Fatty acid (MW 3726.67)	567.14 ± 203.14	1075.56 ± 235.68	0.004
Fatty acid (MW 3741.38)	49.09 ± 28.28	129.70 ± 40.53	0.004
Fumaric acid	58.17 ± 39.37	80.22 ± 36.13	0.362
Galactose	84.29 ± 79.38	129.52 ± 41.02	0.281
Glucaric acid-1,4-lactone	2565.21 ± 905.37	3874.25 ± 656.41	0.025
Glucopyranose	19211.57 ± 17902.65	21947.79 ± 7217.53	0.757
Glucopyranosid	6919.45 ± 10848.44	15656.08 ± 10027.08	0.202
Glucose	73019.83 ± 27237.25	110146.49 ± 14409.74	0.023
Glucose-6-P	22.97 ± 13.53	30.69 ± 12.36	0.353
Glucuronic acid	41.59 ± 23.92	33.79 ± 35.30	0.673
Glutamic acid	363.29 ± 95.52	279.71 ± 80.54	0.156
Glutamine	1804.06 ± 539.35	2010.98 ± 1714.46	0.784
Glutamine (H2O)	259.37 ± 140.08	201.07 ± 52.64	0.405
Glyceric acid	16.20 ± 5.42	17.98 ± 3.91	0.556
Glycerol	3910.45 ± 2838.93	4393.91 ± 1194.51	0.732
Glycerol-3-phosphate	102.23 ± 40.82	115.92 ± 28.96	0.546
Glycine	129.98 ± 47.82	106.49 ± 70.55	0.527
Glycolic acid	344.67 ± 61.14	434.54 ± 84.78	0.071
Gulose	2309.28 ± 1110.04	3299.87 ± 717.15	0.121
Heneicosan-1-ol	6.52 ± 3.73	9.05 ± 9.05	0.546
Heptadecanoic acid	10.88 ± 3.86	11.87 ± 3.52	0.669

Table 32 continued:

Metabolite (normalized peak intensity)	12 E%	48 E%	*p*-value
Hexadecanoic acid	1684.11 ± 149.42	2080.34 ± 145.06	0.002
Hexadecenoic acid (n-1)	17.86 ± 5.51	17.53 ± 5.77	0.924
Hexadecenoic acid (n-2)	226.60 ± 94.52	174.37 ± 35.20	0.275
Hexose	1718.88 ± 559.03	3186.64 ± 703.95	0.004
Histidine	297.60 ± 168.47	248.71 ± 168.54	0.643
Isoascorbic acid	5.02 ± 8.10	16.72 ± 10.83	0.070
Isoleucine	212.48 ± 113.49	247.17 ± 75.37	0.575
konjugierte AS	18.79 ± 12.32	32.07 ± 5.31	0.053
Lactic acid	77763.94 ± 33718.50	112413.91 ± 18306.75	0.071
Leucine	509.38 ± 333.49	574.54 ± 97.27	0.685
Lysine	387.41 ± 202.81	474.96 ± 324.07	0.597
Lyxose	23.92 ± 18.79	10.92 ± 19.52	0.290
Malic acid	669.31 ± 409.36	822.87 ± 246.42	0.483
Maltose	6.41 ± 6.53	12.68 ± 3.72	0.091
Mannose	92696.76 ± 51239.48	136239.66 ± 23191.55	0.115
Mannose-6-phosphate	113.73 ± 39.56	111.80 ± 21.96	0.925
Methionine	421.76 ± 146.19	353.23 ± 141.44	0.452
myo-inositol	727.76 ± 496.29	564.44 ± 196.42	0.509
Octadecanoic acid	814.93 ± 57.17	909.97 ± 110.49	0.098
Octadecanoic acid, 2,3-dihydroxypropylester	37.69 ± 7.31	45.96 ± 11.89	0.190
Ornithine	107.45 ± 35.60	84.53 ± 33.40	0.303
Ornithine-1,5-lactam	7.74 ± 6.06	7.67 ± 6.34	0.985
Orotic acid	3.52 ± 4.30	10.48 ± 7.18	0.077
Pantothenic acid	27.11 ± 20.60	29.14 ± 20.43	0.874
Phenylalanine	810.12 ± 290.68	868.62 ± 67.43	0.673
Phosphoric acid	1143.29 ± 671.77	1237.11 ± 504.43	0.803
phosphoric acid, monomethyl ester	8.02 ± 8.21	13.41 ± 8.64	0.318
Phtalat	27.09 ± 4.48	28.25 ± 4.82	0.688
Proline	424.29 ± 284.28	305.74 ± 142.36	0.421
Proline (CO_2)	109.03 ± 30.87	152.62 ± 85.26	0.271
Pyroglutamic acid	337.45 ± 119.59	359.72 ± 205.26	0.827
Pyrophosphate	339.83 ± 197.66	455.27 ± 121.33	0.286
Pyruvic acid	570.52 ± 349.53	676.66 ± 205.30	0.566
Rhamnose	10.19 ± 2.68	10.68 ± 4.56	0.827
Saccharic acid	10.91 ± 11.83	22.71 ± 13.37	0.155
Serine	1137.83 ± 815.08	839.84 ± 221.17	0.451
Sitosterol	17.68 ± 8.19	19.55 ± 7.05	0.698
Sorbose	199.82 ± 89.70	159.86 ± 96.66	0.495
Sterol	7.49 ± 1.37	12.91 ± 4.08	0.013
Succinic acid	736.49 ± 1101.65	1985.20 ± 1696.28	0.174
Sucrose	12.73 ± 10.96	9.71 ± 10.92	0.659
Sugar alcohol	130.42 ± 75.14	244.31 ± 56.80	0.021
Sulfuric acid	108.72 ± 17.18	127.96 ± 16.30	0.091

Table 32 continued:

Metabolite (normalized peak intensity)	12 E%	48 E%	*p*-value
Tetradecanoic acid	58.98 ± 22.97	36.93 ± 3.91	0.065
Threitol	29.11 ± 5.28	31.68 ± 6.02	0.471
Threonic acid	18.00 ± 14.84	38.28 ± 36.09	0.238
Threonine	1269.70 ± 453.96	1290.29 ± 580.94	0.949
Tryptophan	2576.33 ± 746.46	2253.19 ± 232.72	0.380
Tyrosine	1719.01 ± 857.00	1739.89 ± 706.89	0.966
Uracil	24.61 ± 15.60	28.84 ± 9.57	0.612
Urea	8835.17 ± 744.67	8916.39 ± 701.52	0.857
Uric acid	411.48 ± 295.93	749.96 ± 430.19	0.157
Uridine	35.44 ± 30.39	78.09 ± 44.90	0.093
Valine	1715.43 ± 516.55	1713.53 ± 135.77	0.994
Xylose	19.40 ± 9.22	14.05 ± 2.92	0.247
α-adenosin	5.27 ± 8.48	12.81 ± 20.07	0.422
α-Tocopherol	298.39 ± 80.57	417.93 ± 93.50	0.049
1,3-Propandiol	41.65 ± 24.73	30.39 ± 13.12	0.386
1-methyl-tryptamine	38.41 ± 8.47	46.05 ± 13.69	0.285
2-amino-adipic acid	79.98 ± 19.41	82.86 ± 9.74	0.771
2-amino-butanoic acid	45.06 ± 25.66	41.72 ± 8.89	0.789
2-hydroxy-butanoic acid	193.64 ± 188.27	203.40 ± 66.74	0.915
2-hydroxy-glutaric acid	9.73 ± 10.41	13.69 ± 8.42	0.513
2-oxo-butanoic acid	12.24 ± 4.70	9.84 ± 1.36	0.302
2-oxo-gluconic acid	3672.25 ± 4153.69	11686.62 ± 4028.06	0.010
2-oxo-glutaric acid	131.03 ± 50.54	175.55 ± 45.65	0.163
2-oxo-gulonic acid	306.89 ± 549.03	737.96 ± 616.17	0.251
2-oxo-isocaproic acid	20.48 ± 12.82	19.27 ± 3.52	0.843
2-oxo-isovaleric acid	40.62 ± 23.92	37.01 ± 14.79	0.777
2-oxo-valeric acid	30.00 ± 7.13	21.37 ± 12.30	0.178
3-hydroxy-butanoic acid	2964.88 ± 1780.97	3132.34 ± 1326.29	0.866
3-Iodo-tyrosine	18.73 ± 5.01	23.76 ± 10.54	0.324
3-Methyl-2-oxopentanoic acid	15.12 ± 6.03	8.37 ± 7.76	0.138
4-hydroxy-butanoic acid	20.41 ± 12.23	31.80 ± 11.02	0.143
4-hydroxy-proline	56.97 ± 20.61	37.82 ± 14.63	0.116
9,12-Octadecadienoic acid (Z,Z)	727.16 ± 118.51	561.31 ± 53.84	0.018
9-Octadecenoic acid (Z)	165.06 ± 40.64	149.86 ± 14.85	0.451

Data are presented as mean ± SD. *P*-value obtained by unpaired t-test.

Suppl. Table 33: Amino acid concentrations in kidney of male mice after twelve weeks dietary intervention (study 2)

Wildtype animals were fed a control (11 energy% of fat, n=9) or high-fat diet (60 energy% of fat, n=8) for 12 weeks and amino acid concentrations were analyzed via LC-MS/MS.

Amino acid (µmol/g protein)	11 E%	60 E%	*p*-value	*p*-value adj
α-aminoadipidate	1.33 ± 0.55	0.96 ± 0.20	0.093	0.27
α-aminobutyrate	0.78 ± 0.31	0.41 ± 0.12	0.007	0.116
Alanine	75.33 ± 21.15	72.29 ± 13.26	0.732	0.832
Anserine	0.88 ± 0.38	0.61 ± 0.11	0.068	0.27
Arginine	4.31 ± 0.82	4.01 ± 0.64	0.417	0.564
Asparagine	5.70 ± 0.91	5.54 ± 0.81	0.724	0.832
Aspartate	38.74 ± 10.82	50.12 ± 12.40	0.061	0.27
β-Alanine	0.51 ± 0.42	0.61 ± 0.41	0.651	0.775
β-aminoisobutyrate	0.14 ± 0.08	0.14 ± 0.04	0.904	0.922
Carnosine	0.42 ± 0.13	0.22 ± 0.04*	0.001	0.03
Citrulline	0.48 ± 0.13	0.55 ± 0.09	0.222	0.427
Cystathionine	0.41 ± 0.17	0.27 ± 0.10	0.066	0.27
Cystine	50.09 ± 13.39	48.57 ± 11.02	0.803	0.873
Ethanolamine	10.82 ± 1.43	10.23 ± 1.26	0.384	0.55
GABA	1.33 ± 0.55	0.95 ± 0.23	0.094	0.27
Glutamate	196.41 ± 56.28	161.73 ± 24.35	0.128	0.292
Glutamine	43.99 ± 9.36	31.42 ± 5.38	0.005	0.113
Glycine	182.61 ± 34.80	143.37 ± 17.22	0.011	0.142
Histidine	4.46 ± 0.82	4.47 ± 0.94	0.979	0.979
Homocitrulline	0.09 ± 0.06	0.09 ± 0.05	0.857	0.912
Hydroxylysine	0.12 ± 0.07	0.10 ± 0.08	0.641	0.775
Hydroxyproline	2.73 ± 0.39	3.33 ± 0.73	0.047	0.27
Isoleucine	7.14 ± 1.69	6.10 ± 1.13	0.162	0.353
Leucine	13.17 ± 2.89	10.82 ± 2.40	0.09	0.270
Lysine	10.41 ± 2.28	8.83 ± 1.37	0.109	0.271
Methionine	3.45 ± 1.11	2.43 ± 0.98	0.066	0.27
Ornithine	1.46 ± 0.57	1.75 ± 0.46	0.278	0.49
P-Ethanolamine	59.28 ± 10.06	55.23 ± 8.27	0.383	0.550
Phenylalanine	4.30 ± 0.76	3.85 ± 0.65	0.215	0.427
Proline	7.88 ± 2.40	7.37 ± 1.21	0.60	0.755
P-Serine	0.08 ± 0.04	0.06 ± 0.03	0.382	0.550
Sarcosine	0.11 ± 0.05	0.09 ± 0.04	0.385	0.550
Serine	13.95 ± 2.87	12.50 ± 2.47	0.284	0.49
Taurine	230.87 ± 47.22	255.56 ± 41.72	0.274	0.49
Threonine	12.24 ± 2.68	12.07 ± 2.42	0.894	0.922
Tryptophan	1.67 ± 0.30	1.44 ± 0.20	0.09	0.27
Tyrosine	5.62 ± 1.46	5.15 ± 1.10	0.464	0.61
Valine	13.86 ± 3.15	11.46 ± 2.41	0.102	0.27
1-M-Histidine	1.68 ± 0.36	1.91 ± 0.21	0.123	0.292
3-M-Histidine	0.62 ± 0.18	0.93 ± 0.43	0.066	0.270
Sum	1009.68 ± 208.32	937.77 ± 132.90	0.417	0.564

Data are presented as mean ± SD. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction.

Suppl. Table 34: Amino acid concentrations in small intestine of male mice after twelve weeks dietary intervention (study 2)

Wildtype animals were fed a control (11 energy% of fat, n=9) or high-fat diet (60 energy% of fat, n=8) for 12 weeks and amino acid concentrations were analyzed via LC-MS/MS.

Amino acids (µmol/g protein)	11 E%	60 E%	*p*-value	*p*-value adj.
α-aminoadipate	0.76 ± 0.16	0.87 ± 0.33	0.375	0.868
α-aminobutyrate	2.23 ± 1.05	2.88 ± 0.47	0.129	0.856
Alanine	256.08 ± 32.03	247.48 ± 37.30	0.616	0.987
Anserine	0.18 ± 0.18	0.16 ± 0.06	0.797	0.987
Arginine	89.73 ± 21.39	89.20 ± 25.29	0.963	0.999
Argininosuccinate	4.17 ± 1.45	2.53 ± 1.14	0.022	0.77
Asparagine	69.88 ± 9.98	64.47 ± 10.12	0.285	0.856
Aspartate	54.27 ± 8.41	55.16 ± 8.80	0.836	0.987
Citrulline	5.77 ± 0.81	6.97 ± 1.81	0.091	0.856
Cystine	28.79 ± 5.32	29.85 ± 1.80	0.600	0.987
Ethanolamine	46.76 ± 5.30	41.20 ± 4.67	0.038	0.770
GABA	2.01 ± 0.44	2.06 ± 0.54	0.846	0.987
Glutamate	165.48 ± 21.71	175.64 ± 37.26	0.497	0.987
Glutamine	68.88 ± 15.57	76.79 ± 14.39	0.296	0.856
Glycine	205.98 ± 19.04	206.15 ± 21.07	0.986	0.999
Histidine	29.47 ± 5.74	26.85 ± 4.43	0.313	0.856
Hydroxyproline	0.95 ± 0.09	1.00 ± 0.21	0.511	0.987
Isoleucine	62.15 ± 10.85	55.58 ± 10.97	0.235	0.856
Leucine	119.89 ± 19.90	114.31 ± 21.20	0.584	0.987
Lysine	117.92 ± 21.74	116.99 ± 23.04	0.933	0.999
Methionine	35.70 ± 7.69	31.49 ± 7.31	0.267	0.856
Ornithine	19.06 ± 2.61	20.76 ± 4.05	0.317	0.856
P-Ethanolamine	8.86 ± 2.67	7.65 ± 5.28	0.553	0.987
Phenylalanine	70.29 ± 14.04	71.49 ± 14.72	0.866	0.987
Proline	87.39 ± 20.82	79.35 ± 14.99	0.381	0.868
Sarcosine	0.12 ± 0.05	0.11 ± 0.03	0.714	0.987
Serine	129.72 ± 19.68	126.17 ± 17.52	0.701	0.987
Taurine	319.99 ± 25.92	325.37 ± 36.57	0.729	0.987
Threonine	85.11 ± 12.65	82.44 ± 12.18	0.666	0.987
Tryptophan	13.63 ± 2.72	12.31 ± 2.36	0.307	0.856
Tyrosine	60.33 ± 10.42	60.33 ± 11.91	0.999	0.999
Valine	101.21 ± 14.28	90.88 ± 16.47	0.186	0.856
Sum	2262.76 ± 257.64	2224.49 ± 338.67	0.795	0.987

Data are presented as mean ± SD. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction.

Suppl. Table 35: Liver amino acid concentrations of male mice after twelve weeks dietary intervention (study 1)

Wildtype animals were fed a control (10 energy% of fat, n=12) or high-fat diet (45 energy% of fat, n=12) for 12 weeks and amino acid concentrations were analyzed via LC-MS/MS.

Amino acids (µmol/g protein)	10 E%	45 E%	*p*-value	*p*-value adj.
α-aminoadipate	3.58 ± 1.99	1.76 ± 0.63*	0.019	0.050
α-aminobutyrate	3.33 ± 1.10	2.59 ± 0.41	0.006	0.028
Alanine	93.93 ± 15.05	102.94 ± 18.55	0.039	0.068
Asparagine	3.79 ± 0.77	3.34 ± 0.68	0.143	0.178
Aspartate	4.15 ± 1.22	3.82 ± 1.02	0.478	0.524
β-Alanine	2.24 ± 0.87	1.17 ± 0.33**	0.001	0.007
β-aminoisobutyrate	0.45 ± 0.10	0.30 ± 0.04**	<0.001	0.003
Citrulline	0.43 ± 0.15	0.56 ± 0.17	0.058	0.086
Cystathionine	0.27 ± 0.09	0.28 ± 0.09	0.76	0.777
Cystine	0.18 ± 0.08	0.14 ± 0.04	0.166	0.202
Ethanolamine	1.80 ± 0.66	1.28 ± 0.34	0.026	0.057
GABA	0.50 ± 0.08	0.38 ± 0.06**	<0.001	0.007
Glutamate	35.04 ± 6.43	32.25 ± 3.20	0.192	0.227
Glutamine	90.42 ± 9.55	106.17 ± 13.03*	0.003	0.016
Glycine	62.24 ± 4.67	55.32 ± 4.77*	0.002	0.012
Histidine	12.56 ± 0.95	12.87 ± 1.33	0.512	0.549
Homocitrulline	0.07 ± 0.05	0.06 ± 0.03	0.524	0.549
Hydroxyproline	0.53 ± 0.10	0.36 ± 0.09**	<0.001	0.005
Isoleucine	4.92 ± 0.49	4.35 ± 0.83	0.051	0.079
Leucine	10.70 ± 2.08	8.69 ± 1.69*	0.016	0.047
Lysine	12.37 ± 1.59	10.37 ± 1.62*	0.006	0.028
Methionine	1.74 ± 0.51	1.22 ± 0.37*	0.01	0.035
Ornithine	4.98 ± 0.87	3.68 ± 1.03*	0.003	0.016
P-Ethanolamine	7.68 ± 1.35	6.47 ± 1.06*	0.022	0.053
Phenylalanine	3.54 ± 0.88	2.89 ± 0.66	0.051	0.079
Proline	5.98 ± 0.76	5.23 ± 0.73*	0.022	0.053
Sarcosine	0.33 ± 0.10	0.51 ± 0.18*	0.009	0.033
Serine	10.91 ± 3.17	7.64 ± 2.27*	0.008	0.033
Taurine	207.74 ± 42.16	248.76 ± 42.38	0.027	0.057
Threonine	7.96 ± 1.65	6.61 ± 1.49	0.047	0.078
Tryptophan	1.10 ± 0.08	1.01 ± 0.11	0.028	0.057
Tyrosine	5.24 ± 0.87	4.29 ± 0.84*	0.013	0.041
Valine	9.06 ± 1.02	8.24 ± 1.40	0.117	0.159
1-M-Histidine	0.71 ± 0.12	0.61 ± 0.07*	0.018	0.050
Sum	610.98 ± 54.02	646.59 ± 56.05	0.127	0.164

Data are presented as mean ± SD. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction.

Suppl. Table 36: Liver amino acid concentrations of male mice after twelve weeks dietary intervention (study 2)

Wildtype animals were fed a control (11 energy% of fat, n=9) or high-fat diet (60 energy% of fat, n=8) for 12 weeks and amino acid concentrations were analyzed via LC-MS/MS.

Amino acid (µmol/g protein)	11 E%	60 E%	*p*-value	*p*-value adj
α-aminoadipate	1.15 ± 0.74	0.56 ± 0.14	0.042	0.073
α-aminobutyrate	0.96 ± 0.37	0.50 ± 0.12*	0.005	0.017
Alanine	99.31 ± 16.13	104.34 ± 17.28	0.544	0.635
Asparagine	4.65 ± 0.46	3.91 ± 0.51*	0.007	0.02
Aspartate	4.23 ± 0.87	3.66 ± 0.33	0.106	0.162
β-Alanine	1.22 ± 0.35	1.19 ± 0.41	0.887	0.89
β-aminoisobutyrate	0.15 ± 0.04	0.08 ± 0.02**	<0.001	0.011
Citrulline	0.42 ± 0.11	0.36 ± 0.04	0.133	0.197
Cystathionine	0.47 ± 0.22	0.27 ± 0.11	0.037	0.072
Cystine	0.35 ± 0.13	0.35 ± 0.13	0.89	0.89
Ethanolamine	0.55 ± 0.16	0.49 ± 0.13	0.47	0.577
GABA	0.52 ± 0.18	0.44 ± 0.08	0.256	0.343
Glutamate	28.89 ± 10.12	27.96 ± 1.97	0.802	0.881
Glutamine	73.93 ± 14.60	86.57 ± 13.04	0.081	0.129
Glycine	48.20 ± 6.91	37.94 ± 3.42*	0.002	0.012
Histidine	10.65 ± 1.85	10.07 ± 1.20	0.464	0.577
Homocitrulline	0.11 ± 0.04	0.05 ± 0.03*	0.003	0.013
Hydroxyproline	0.50 ± 0.10	0.38 ± 0.06*	0.011	0.03
Isoleucine	6.55 ± 0.93	5.08 ± 0.92*	0.005	0.017
Leucine	11.63 ± 1.94	9.22 ± 1.46*	0.012	0.03
Lysine	12.32 ± 1.51	9.90 ± 1.16*	0.002	0.012
Methionine	0.64 ± 0.16	0.55 ± 0.10	0.191	0.274
Ornithine	6.18 ± 1.20	4.55 ± 0.62*	0.004	0.014
P-Ethanolamine	4.58 ± 1.18	4.49 ± 0.40	0.842	0.883
Phenylalanine	4.21 ± 0.48	3.52 ± 0.56*	0.015	0.035
Proline	8.19 ± 1.99	6.50 ± 1.04	0.048	0.079
Sarcosine	1.76 ± 1.26	0.75 ± 0.30	0.042	0.073
Serine	7.53 ± 0.79	5.85 ± 0.83*	0.001	0.012
Taurine	187.45 ± 19.26	208.79 ± 15.54	0.025	0.054
Threonine	7.78 ± 1.07	7.16 ± 0.96	0.288	0.316
Tryptophan	1.16 ± 0.12	0.95 ± 0.12*	0.002	0.012
Tyrosine	4.70 ± 0.46	4.08 ± 0.69	0.042	0.073
Valine	12.72 ± 1.61	9.56 ± 1.57*	0.001	0.012
1-M-Histidine	0.43 ± 0.10	0.46 ± 0.06	0.547	0.866
Sum	554.21 ± 67.85	560.67 ± 41.59	0.8119	0.881

Data are presented as mean ± SD. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction.

Suppl. Table 37: Amino acid concentrations in skeletal muscle of male mice after twelve weeks dietary intervention (study 2)

Wildtype animals were fed a control (11 energy% of fat, n=9) or high-fat diet (60 energy% of fat, n=8) for 12 weeks and amino acid concentrations were analyzed via LC-MS/MS.

Amino acids (µmol/g protein)	11 E%	60 E%	*p*-value	*p*-value adj
α-aminoadipate	0.15 ± 0.04	0.11 ± 0.04	0.068	0.185
α-aminobutyrate	1.27 ± 0.28	0.96 ± 0.25	0.032	0.093
Alanine	88.15 ± 4.06	89.19 ± 13.28	0.825	0.89
Anserine	107.99 ± 7.48	98.09 ± 9.33	0.028	0.089
Arginine	4.86 ± 0.84	3.86 ± 0.72	0.02	0.075
Asparagine	2.73 ± 0.23	3.75 ± 0.52**	<0.001	0.002
Aspartate	1.48 ± 0.20	1.81 ± 0.32	0.02	0.075
Carnosine	70.41 ± 7.55	56.24 ± 4.77**	<0.001	0.005
Citrulline	2.55 ± 0.30	3.07 ± 0.43	0.011	0.057
Cystathionine	5.38 ± 1.16	4.45 ± 0.83	0.08	0.206
Ethanolamine	0.81 ± 0.14	0.93 ± 0.18	0.153	0.314
GABA	0.18 ± 0.07	0.19 ± 0.08	0.866	0.911
Glutamate	16.90 ± 1.77	15.63 ± 1.70	0.153	0.314
Glutamine	44.05 ± 5.08	46.31 ± 3.09	0.293	0.501
Glycine	46.06 ± 4.38	47.54 ± 5.09	0.53	0.658
Histidine	3.42 ± 0.47	3.12 ± 0.53	0.228	0.424
Hydroxyproline	4.60 ± 0.74	3.67 ± 0.51	0.009	0.055
Isoleucine	2.86 ± 0.43	2.95 ± 0.30	0.646	0.756
Leucine	4.58 ± 0.59	4.42 ± 0.38	0.527	0.658
Lysine	19.66 ± 4.27	13.47 ± 2.64*	0.003	0.025
Methionine	1.75 ± 0.37	1.95 ± 0.49	0.349	0.536
Ornithine	0.86 ± 0.19	0.87 ± 0.19	0.924	0.924
P-Ethanolamine	2.27 ± 0.47	2.20 ± 0.16	0.669	0.762
Phenylalanine	2.06 ± 0.24	2.02 ± 0.26	0.794	0.879
Proline	11.19 ± 2.00	9.73 ± 1.58	0.118	0.285
Sarcosine	0.17 ± 0.02	0.10 ± 0.02***	<0.001	<0.001
Serine	9.82 ± 1.29	12.37 ± 1.97**	0.006	0.042
Taurine	320.36 ± 24.54	329.02 ± 22.83	0.465	0.615
Threonine	9.45 ± 0.73	10.30 ± 1.49	0.152	0.314
Tryptophan	0.61 ± 0.13	0.55 ± 0.08	0.349	0.536
Tyrosine	2.92 ± 0.40	3.06 ± 0.68	0.621	0.749
Valine	8.26 ± 0.91	7.63 ± 1.23	0.24	0.427
Sum	797.83 ± 40.57	779.54 ± 54.78	0.442	0.605

Data are presented as mean ± SD. *P*-value obtained by unpaired t-test and adjusted with Benjamini-Hochberg correction.

B Abbreviations

GENERAL ABBREVATIONS

°C	degree celcius
µlU	microliter units
µmol	micromol
a.m.	ante meridiem
AA	amino acids
acyl-CoAs	activated fatty acids
Arg I	arginase I
Arg II	arginase II
ASL	arginine succinate-lyase
ASS	arginine succinate-synthetase
ATP	adenosine triphosphate
BCAA	branched-chain amino acids
BCAT	branched-chain amino acid aminotransferase
BCKA	branched-chain α-keto acids
BCKD	branched-chain α-keto acid dehydrogenase complex
BMI	body-mass index
C	control
CACT	carnitine:acylcarnitine translocase
CET	cycling ergometer test
CN	correlation network
CP	carbamoyl-phosphate
CPT2	carnitine palmitoyltransferase 2
CV	coefficient of variation
DIO	diet-induced obesity
dl	deciliter
EDTA	ethylendiaminetetraacetic acid
E%	energy%
F12	12 hours fasting group
F24	24 hours fasting group
FA	fatty acids
FC	fold-change
FYVE	Fab1p, YOTB, Vac1p, EEA1, zinc finger domain
g	gram
GAA	glucogenic amino acids
GC-MS	gas chromatography
h	hours
HCA	hierarchical clustering analysis
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
HF	high-fat
HuMet	Human Metabolome
hVps34	human vacuolar protein sorting 34
i.e.	id est
IRS1	insulin receptor substrate 1
iTRAQ	isobaric tag for relative and absolute quantification
kcal	kilocalories
kg	kilogram
K_M	Michaelis constant
l	liter
LAT1	L-type amino acid transporter 1
LAT2	L-type amino acid transporter 2
LC-MS	liquid chromatography
LC-MS/MS	liquid chromatography tandem mass spectrometry
m	meter
mg	milligram
M	molecular mass
MeOH	methanol
min	minutes

mmol	millimol
mosm	milliosmol
MS	mass spectrometry
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide
mTOR	mammalian target of rapamycin
mTORC1	mammalian target of rapamycin complex 1
mTORC2	mammalian target of rapamycin complex 2
NEFA	non-esterified fatty acids
nmol	nanomol
NMR	nuclear magnetic resonance
NO	nitric oxide
NOS	nitric oxide synthase
npi	normalized peak intensity
NuGO	nutrigenomics organization
OGTT	oral glucose tolerance test
OH	hydroxy
OLTT	oral lipid tolerance test
OTC	ornithine carbamoyl transferase
p.m.	past meridian
P5C	pyrroline-5-carboxylate
PCA	principal component analysis
PDH	pyruvate dehydrogenase complex
PDK1	phosphoinositide-dependent kinase
PEMT	phosphatidylethanolamine N-methyltransferase
PI3-kinase	phosphatidylinositol 3 kinase
PI3P	phosphatidylinositol 3-phosphate
PIP	phosphatidylinositol phosphate
PIP_2	phosphatidylinositol (4,5)-tripohsphate
PIP_3	phosphatidylinositol (3,4,5)-tripohsphate
PKB	protein kinase B
PL	phospholipid
pmol	picomol
ppm	parts per million
PPS3	proof of principle study 3
PX	phosphoinositide-binding structural doman
Rheb	ras homolog enriched in brain
S6K1	p70 S6 kinase 1
SD	standard deviation
SEM	standard error of mean
SLD	standard liquid diet
suppl.	supplementary
t	time
T2DM	type 2 diabetes mellitus
TCA	tricarboxylic acid cycle
TG	triglyceride
TSC1	tuberous sclerosis protein 1
TSC2	tuberous sclerosis protein 2
u.a.	unter anderem
v/v	volume/volume
VLDL	very low density lipoproteins
VO_2max	maximal aerobic capacity
vs.	versus
W	watt
WHO	world health organization
ZDF	zucker diabetic fatty

ABBREVIATIONS OF AMINO ACIDS

1MHis	1-methyl-histidine
3MHis	3-methyl-histidine
AAD	α-aminoadipate
ABU	α-aminobutyrate
ALA	alanine

ASN	asparagine
ARG	arginine
ASA	argininosuccinate
bAIB	β-aminoisobutyrate
bAla	β-alanine
CAR	carnosine
CTH	cystathionine
CYS	cystine
CIT	citrulline
EtN	ethanolamine
GLN	glutamine
GLU	glutamate
GLY	glycine
HCIT	homocitrulline
HIS	histidine
HYP	hydroxyproline
ILE	ieucine
LEU	leucine
LYS	lysine
MET	methionine
ORN	ornithine
PEtN	P-ethanolamine
PHE	phenylalanine
PRO	proline
SAR	sarcosine
SER	serine
TAU	taurine
THR	threonine
TRP	tryptophan
TYR	tyrosine
VAL	valine

ABBREVIATIONS OF METABOLTES MEASURED VIA ^{1}H NMR

2-PP	2-phenylpropionate
2-OHB	2-hydroxy-butyrate
3-OHB	3-hydroxy-butyrate
AAc	acetoacetate
Crea	creatine
Lac	lactate
Pyr	pyruvate
OAc	acetate

ABBREVIATIONS OF ACYLCARNITINE SPECIES

C0	free carnitine
C2	acetylcarnitine
C3	propionylcarnitine
C3:1	propenoylcarnitine
C3-OH	OH-propionylcarnitine
C4	butyrylcarnitine
C4:1	butenylcarnitine
C4-OH	OH-butyrylcarnitine
C5	valerylcarnitine
C5:1	tiglylcarnitine
C5:1-DC	glutaconylcarnitine
C5-DC	glutarylcarnitine
C5-M-DC	methylglutarylcarnitine
C5-OH	OH-valerylcarnitine
C6	hecanoylcarnitine
C6:1	hexenoylcarnitine
C7-DC	pimelylcarnitin
C8	octanoylcarnitine
C9	nonaylcarnitine

C10	decanoylcarnitine
C10:1	decenoylcarnitine
C10:2	decadienylcarnitine
C12	dodecanoylcarnitine
C12:1	dodecenoylcarnitine
C12-DC	dodecanedioylcarnitine
C14	tetradecanoylcarnitine
C14:1	tetradecenoylcarnitine
C14:1-OH	OH-tetradecenoylcarnitine
C14:2	tetradecadienylcarnitine
C14:2-OH	OH-tetradecadienylcarnitine
C16	hexadecanoylcarnitine
C16:1	hexadecenoylcarnitine
C16:1-OH	OH-hexadecenoylcarnitine
C16:2	hexadecadienylcarnitine
C16:2-OH	OH-hexadecadienylcarnitine
C16-OH	OH-hexadecanoylcarnitine
C18	octadecanoylcarnitine
C18:1	octadecenoylcarnitine
C18:1-OH	OH-octadecenoylcarnitine
C18:2	octadecadienylcarnitine

.

Danksagung

Zu dieser Arbeit gehören viele Wegbegleiter, „Helfer“ und Unterstützer, die auf sehr unterschiedliche Art und Weise dazu beigetragen haben, dass diese Arbeit entstanden ist. Der DANK gilt

Frau Prof. Hannelore Daniel: Sie haben mir die Möglichkeit gegeben, diese Doktorarbeit zu meiner Doktorarbeit zu machen. Danke für Ihre Begeisterung für die Metabolom-Daten, die langen Diskussion und das Vertrauen, dass Sie mir zugesprochen haben - auch durch die Überlassung vieler kleiner Nebenaufgaben und Projekte. Herzlichen Dank auch an Herrn Prof. Hauner und Herrn Prof. Klingenspor für die Beurteilung meiner Arbeit.

Dr. Isabel Rubio-Aliaga. Isabel du hast mir durch die Betreuung bis zur „Halbzeit“ meiner Doktorarbeit den bestmöglichsten Start beschert! Danke, dass du mich motiviert und gefördert hast, mit mir diskutiert hast und mich zum Erlernen von R - trotz großer, anfänglicher Skepsis - ermutigt hast!

Ronny Scheundel, Johanna Welzhofer, Barbara Gelhaus, Alexander Haag. Ihr wart mir bei den unterschiedlichsten Experimente, Laborfragen und – problemen eine große Hilfe!

Allen, die mit Anregungen, Kritik und Ideen zu dieser Arbeit beigetragen haben!

dem NuGO-PPS Team und dem HuMet-Konsortium. Es war toll in diesen Projekten involviert gewesen zu sein! Danke vor allem an Susanne Krug, für die vielen tollen Gespräche und die Freundschaft, die sich entwickelt hat!

JEDEM Mitglied der AG Daniel – ohne euer Lachen, eure gute Laune, euren Kuchen, den Gesprächen, Diskussionen usw. wäre die Zeit am Institut nur halb so schön! Besonderer Dank gilt Laure, Ramona, Kerstin, Gregor, Pieter und Christoph. Danke auch an meine Bürokolleginnen Veronika und Martina für die Plaudereien zwischendurch!

Tamara Zietek und Dorothea Wörner. Danke für euer offenes Ohr in jeder Lebenslage!

Anne Nässl, Jacqueline Benner und Mena Eidens. Ihr seid mir sehr ans Herzen gewachsen und ich bin sehr froh, dass ihr mir jeder Zeit mit Rat und Tat zur Seite steht. Egal ob Arbeit oder Privat!

Meiner Familie: Mama, Papa, Jürgen, Andrea, Patrick und meiner Schwägerin Ying. Euch gilt der größte DANK, denn ohne Euch hätte ich das nicht geschafft! Ich weiß, dass ich mich immer auf euch verlassen kann! DANKE für eure Liebe und eure Unterstützung!

List of scientific publications

Selected poster presentations

2011 DECHEMA “Trends in metabolomics” – Frankfurt, Germany
Assessing the dynamics of changes in the human plasma amino acid pool

2010 NuGO Week – Glasgow, United Kingdom
Amino acid profiling as a toll to assess metabolic plasticity in humans

International Pediatric Biomarker Symposium – Innsbruck, Austria
Citrulline may be a marker of insulin resistance in high-fat fed mice

2009 NuGO Week – Montecatini Terme, Italy
High fat diets alter hepatic amino acid and lipid metabolism in mice

2008 NuGO Week – Potsdam, Germany
Amino acid profiling in plasma for biomarker discovery in the evelopment of insulin resistance in C57BL/6J mice

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Erklärung

Hiermit versichere ich, dass die vorliegende Arbeit

Metabolic interrelationships of amino acid metabolism in health and disease

selbständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die den benutzten Quellen wörtlich und inhaltlich entnommenen Stellen sind als solche kenntlich gemacht.

Diese Arbeit hat in gleicher oder ähnlicher Form noch keiner anderen Prüfungsbehörde vorgelegen.

Freising, den

Manuela Sailer