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Pathophysiological aspects of metabolism in patients with inflammatory bowel diseases and in patients with liver cirrhosis: Relevance for clinical nutrition

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von
Dr. rer. nat. Luzia Valentini
geboren am 19.01.1965 in Brixlegg, Österreich

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Dekanin: Univ.-Prof. Dr. Annette Grüters-Kieslich
1. Gutachter: Univ.-Prof. Dr. Hannelore Daniel, München
2. Gutachter: Univ.-Prof. Dr. Wilfred Druml, Wien
3. Gutachter: Univ.-Prof. Dr. Gabriele Stangl, Halle

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2.5	**Valentini L**, Schuetz T, Omar A, Gläser S, Kasim E, Nowotny P, Kroencke T, Ockenga J. *Abnormal plasma peptide YY(3-36) levels in patients with liver cirrhosis.* Nutrition 2011 Sept; **27**(7-8):880-4	71-75

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List of abbreviations

5-ASA	5-aminosalicylic acid
AI	adequate intake
ALT	alanine aminotransferase
art	arterial
AST	aspartate aminotransferase
AUC	area under the curve
AZA	azathioprine
BA	bile acids
BCM	body cell mass
BIA	bioelectrical impedance analysis
BMI	body mass index
β-weight	standardized regression coefficient
CA	cholic acid
CAI	colitis activity index
cAMP	cyclic adenosine monophosphate
CCK	cholecystokinin
CD	Crohn's disease
CDAI	Crohn's disease activity index
CDC	chenodeoxycholic acid
CON	controls
CRP	C-reactive protein
CT	computed tomography
D2	type 2 iodothyronine deiodinase
DC	deoxycholic acid
DHA	docosahexaenoic acid
dLC	decompensated liver cirrhosis
DPA	docosapentaenoic acid
EAR	estimated average requirement
ECM	extracellular water
EE	energy expenditure
EFSA	European Food and Safety Agency
EPA	eicosapentaenoic acid
FA	fatty acids
FFQ	food frequency questionnaire
FM	fat mass

fT3	free triiodothyronine
fT4	free thyroxine
GCA	glycocholic acid
GCDC	glycochenodeoxycholic acid
GDC	glycodeoxycholic acid
GLP-1	glucagon-like peptide 1
GI	gastrointestinal
GPR	G-protein coupled receptor
hBAT	human brown adipose tissue
HOMA	homeostasis model assessment
IBD	inflammatory bowel diseases
ICAM-1	intercellular adhesion molecule 1
IL-6	interleukin-6
INR	international normalized ratio
LC	liver cirrhosis
LBM	lean body mass
mes	mesenteric
MTX	methotrexate
NA	not analysed
NFκB	nuclear factor kappa B
NPY	neuropeptide Y
PBMC	peripheral blood mononuclear cells
PUFA	polyunsaturated fatty acid
PYY	peptide YY
PGE_2	prostaglandin E_2
QOL	quality of life
R	multiple correlation coefficient
R^2 adj	adjusted standardized regression coefficient
RBP-4	retinol-binding protein-4
RCT	randomised controlled trial
RDA	recommended dietary allowance
REE	resting energy expenditure
RQ	respiratory quotient
SCFA	short chain fatty acids
SGA	subjective global assessment
T3	3,5,3´-triiodothyronine
T4	thyroxine

TBW	total body water
TCA	taurocholic acid
TCDC	taurochenodeoxycholic acid
TDC	taurodeoxycholic acid
TIP(S)S	transjugular intrahepatic portosystemic (stent) shunt
TNF α	tumor necrosis factor α
TSH	thyroid stimulating hormone
UL	tolerable upper intake level
UC	ulcerative colitis
VCAM-1	vascular cell adhesion molecule 1
ven	venous
VCO_2	carbon dioxide production
VO_2	oxygen consumption

1 Introduction

Any type of disease inevitably alters metabolism at many levels. Some changes even persist when metabolism is corrected by pharmacological interventions. Pathophysiological alterations in metabolism may potentially affect the requirements for food-derived nutrients. Furthermore, bioactive compounds in food may aggravate or potentially counteract pathophysiological alterations. Detailed knowledge on exact metabolic alterations and their underlying molecular and cellular mechanism is thus pivotal for the effectiveness of any nutritional therapy. The present work describes several new aspects of disease-specific mechanisms in patients with inflammatory bowel diseases (IBD) and in patients with liver cirrhosis (LC).

1.1 Inflammatory bowel diseases and the unknown prevalence of malnutrition in clinical remission

IBD are lifelong medical conditions marked by a relapsing-remitting course consisting of two major forms, i.e. Crohn's disease (CD) and ulcerative colitis (UC) (1). IBD are relatively common in developed countries (2) with a traditionally higher incidence in Northern Europe (3) but also steadily increasing numbers in Mediterranean countries (4). With regard to aetiopathogenesis, hyper-responsiveness of the mucosal immune system (1), genetic susceptibility and environmental factors (5) have been discussed. One important environmental factor is nutrition (6).

Malnutrition in active IBD has been thoroughly investigated and affects up to 75% of patients (7). The key characteristics of malnutrition in active disease are weight loss, hypoalbuminaemia, anaemia and deficiencies of selected vitamins (especially vitamin D and vitamin B_{12}) and trace elements (7;8). The aetiology of malnutrition includes anorexia due to gastrointestinal symptoms, intestinal bleeding, malabsorption and maldigestion. Immunosuppressive medication (9;10) can further aggravate malnutrition; azathioprine, for instance, causes nausea and vomiting in about 12% of patients and methotrexate leads to diarrhoea, nausea and vomiting in up to 25% of patients (11).

Malnutrition in quiescent IBD, however, has not yet been adequately investigated. Nevertheless, knowledge on its prevalence, characteristics and dependence on the inflammatory status, disease location and previous or actual medication is necessary for generating nutritional recommendations in future.

So far, nine clinical trials have investigated the nutritional status of patients with IBD in clinical remission (12-20), but four of these trials included up to 33% of patients with active disease (13-15;18). Of the remaining five trials, only one assessed malnutrition by means of validated standard tools (21). The investigators found that 22% of patients have a moderate risk and 7% of patients a severe risk of malnutrition (19). Results on body composition were inconclusive. The majority of trials found decreased parameters for lean body mass (15-18;20), but some investigators concluded lean body mass to be normal (12;19) or even increased (14). Furthermore, three trials reported gender-related differences (13-15), but two others did not show any difference between men and women (17;18). Only three trials included patients with both UC and CD (12;14;15). One trial found a more compromised nutritional status in patients with UC (14), although CD is generally assumed to have more severe effects on the nutritional status than UC. We presumed that reasons for these inconsistencies were the small samples sizes (12;19) ─ particularly with regard to body composition ─, missing body mass indices (BMIs) and lack of matching controls (13;15). Because both lean body mass and fat mass change with varying BMIs and age, we considered the matching of data on sex, age and BMI particularly important for our investigations.

This part of our investigations aimed at evaluating the nutritional status of patients with IBD in clinical remission using an adequate sample size and comparing the data with BMI-matched and age-matched healthy controls. Specifically, we aimed at the detailed assessment of malnutrition, muscle strength, nutritional intake and quality of life. Furthermore, we wanted to detect persisting changes in body composition and relate these changes to systemic and intestinal inflammation.

1.2 Inflammatory bowel diseases and possible associations between adipokines, body fat mass and inflammation

IBD is associated with alterations in fat mass and fat distribution. Previous investigators reported increased visceral fat mass (22) and creeping fat at the intestinal surface of inflamed bowel areas (23;24). Additionally, a fatty deposition in the submucosa of the intestinal wall, the fat halo sign (25), was observed in 17% of patients with CD (26) and an unknown number of patients with UC (25).

The observed accumulation of visceral and mesenteric fat mass in a substantial number of patients with IBD is rather significant, particularly because, through the discovery of

adipokines, the paradigm of adipose tissue has changed from being an inert reservoir to presenting highly active endocrine tissue. Adipokines (adipose tissue cytokines) are soluble mediators derived from adipose tissue and well-known contributors to insulin resistance (27). Recently, much effort has been made to define the interaction between adipose tissue, adipokines, inflammation and immunity (28). Recent data have suggested that the altered expression of adipokines in the mesenteric fat of patients with Crohn's disease (CD) (29;30) may act protectively in disease pathogenesis (31). Our present work on patients with IBD investigates a number of adipokines including leptin, adiponectin, resistin, retinol-binding protein-4 (RBP-4) and visfatin.

Leptin exerts multiple biological effects on a variety of different processes, such as energy metabolism, immune responses, angiogenesis and wound healing. Leptin also has pro-atherogenic and pro-inflammatory properties (32). Recently, the stomach has been identified as an important source of leptin, and there is growing evidence for the diverse functions of leptin in the gastrointestinal tract (33). Leptin is up-regulated in the colonic mucosa (34), and some (36;37) but not all investigations have shown an increase in the creeping fat of patients with IBD (35) as well as in the plasma concentrations of leptin (38).

Adiponectin is the most abundant adipokine with plasma levels ranging from 3 to 30 µg/mL in humans (39), a concentration that is about 1000 times higher than that of leptin. Adiponectin binding to its receptors Adipo R1 and R2 induces fatty acid oxidation, glucose uptake and suppression of gluconeogenesis in muscle and liver, thereby improving insulin sensitivity (39). In addition to its insulin-sensitising effects, adiponectin may alter glucose metabolism by stimulating pancreatic insulin secretion (40). Adiponectin can modulate food intake and energy expenditure during fasting and re-feeding through central nervous mechanisms (41) and has anti-atherogenic and anti-inflammatory properties (42;43). Several mechanisms have been suggested as an explanation of its anti-inflammatory effects, including direct actions on inflammatory cells, effects on the nuclear factor-κB (NF-κB) and interaction with the tumour necrosis factor α (TNF-α) (44). The anti-inflammatory effects of adiponectin can protect against endothelial dysfunction and plaque initiation (39). Several recent investigations have suggested that adiponectin plays a potential role in a variety of inflammatory diseases. Elevated serum adiponectin was reported in inflammatory bowel diseases (45), rheumatoid arthritis (46), lupus erythematosus (47) and cystic fibrosis (48).

Resistin in humans is mainly secreted by peripheral blood mononuclear cells (PBMCs), macrophages and bone marrow cells (49;50). Resistin is involved in inflammatory processes, and its expression is up-regulated by TNF-α and interleukin (IL)-6. Furthermore, resistin has pro-inflammatory properties by activating NFκB inflammatory pathways (50). In humans,

resistin is associated with C-reactive protein (CRP), soluble TNFα receptor-2, IL-6 and lipoprotein-associated phospholipase A2 in several medical conditions (27).

RbP-4 shows the highest correlation among all adipokines to visceral fat mass (51) and is closely linked to insulin resistance in humans (52). *Visfatin* can be considered a new pro-inflammatory adipokine (53) that is more abundantly expressed in visceral fat than in subcutaneous fat and induces the production of IL-1β, TNF-α and IL-6 (53) in $CD14^+$ monocytes. Visfatin may ameliorate insulin resistance (28). So far, RBP-4 and visfatin have not been evaluated in patients with IBD.

This part of our investigations aimed at evaluating adipokines and glucose metabolism in patients with active and inactive IBD and at correlating the results to disease activity, inflammatory parameters, previous and current medication as well as the clinical course of disease over 6 months.

1.3 Bile acids (BA) as potential modulators of energy expenditure in patients with liver cirrhosis

Patients with liver cirrhosis (LC) often present with increased plasma bile acids (BA), mainly because of impaired removal of BA from portal blood due to parenchymal damage and porto-systemic shunts (54). BA in enterohepatic circulation have many established functions, such as lipid digestion, intestinal proteolysis of dietary proteins (55), direct and indirect antimicrobial effects (56), elimination of cholesterol and many more (57), but the function of plasma BA is less clear.

In 2006, Watanabe and colleagues (58) showed that plasma BA can induce energy expenditure (EE) by promoting intracellular thyroid hormone activation in the brown adipose tissue of mice. Watanabes' group additionally conducted in vitro studies with various human tissues and found each receptor and enzyme necessary for BA effects only in skeletal myocytes and brains cells (58). Since brown adipose tissue in rodents and muscle tissue in humans are the main sites of thermogenesis, the authors hypothesised that BA specifically affect thermogenesis but not ATP production. Comments on the article implied that BA in humans most probably act in the postprandial stage or in patients with liver disease who have chronically increased plasma BA anyway (59).

This mechanism described by Watanabe (58) may be relevant for patients with LC and hence increased plasma BA because hypermetabolism has been previously observed in this

patients group (60-64). Peng and colleagues reported a 15% prevalence of hypermetabolism in 268 patients with LC that was neither associated with sex or origin of the disease nor with disease severity, protein depletion, ascites or tumour presence (65). The underlying mechanisms of hypermetabolism in patients with LC are yet unclear.

The underlying mechanism described by Watanabe (58) starts off with BA binding to the G protein-coupled cell-surface receptor TGR5 (66). This binding results in increased intracellular cAMP production and induction of the Dio2 gene, whose gene product is type 2 iodothyronine deiodinase (D2). D2 then converts locally available inactive thyroxine (T4) to active 3,5,3´-triiodothyronine (T3). T3 actives oxidative phosphorylation in the mitochondria of cells, thereby increasing energy expenditure. Critical for the effect is the concomitant presence of TGR5 and D2 in the respective cell. As mentioned earlier, humans have both TGR5 and D2 in skeletal muscle and in brain cells (58). Postprandial concentration of BA has been assumed to be sufficient for stimulating cAMP production and D2 expression (67). Therefore, BA may be hormonal signals, linking food intake to diet-induced increases in the metabolic rate.
The BA pool in humans consists of different BA subgroups that may stimulate intracellular cAMP production differently. Primary BA are synthesised in the liver via the cytochrome P450-mediated oxidation of cholesterol (68). Secondary BA are generated from primary BA via deconjugation and dehydroxylation by intestinal microbiota. Most human BA are conjugated to either glycine (G) or taurine (T). Primary BA comprise cholic acid (CA) and chenodeoxycholic acid (CDC) and their glycine- and taurine conjugates (GCA, GCDC, TCA and TCDC). Secondary BA include mainly deoxycholic acid (DCA) with small amounts of lithocholic acid (LCA) and ursodeoxycholic acid (UDCA) as well as their glycine- and taurine-conjugates (GDCA, GLCA, GUDCA, TDCA, TLCA, TUDCA). About 95% of BA are reabsorbed from the intestine and returned to the liver to be secreted again in the bile, thereby completing enterohepatic circulation (69).

This part of our investigations aimed at evaluating possible associations between plasma BA (total and subgroups) and energy expenditure in patients with LC in fasting and postprandial conditions compared to healthy controls.

1.4 Bile acids and leptin as potential players in glucose metabolism in patients with liver cirrhosis

Insulin resistance is a common condition in patients with liver cirrhosis (LC) (70) with a reported incidence of glucose intolerance between 60% and 80% depending on the degree

of liver damage (71). Interestingly and counterintuitively, hepatogenous insulin resistance is mainly characterised by peripheral insulin resistance, i.e. in the skeletal muscle (72), whereas uptake of glucose in the liver is normal or even enhanced (72). Peripheral glucose disposal in patients with LC amounts to approximately 50% of that reported for healthy subjects (73;74).

A growing body of evidence exists that BA can positively affect glucose metabolism (75-80). For example, the BA-triggered secretion of glucagon-like peptide 1 (GLP-1) from murine enteroendocrine cells (GLUTag) is dependent on the BA receptor TGR5 (81). In a trial with mice, the BA-TGR5 molecular mechanism led to increased GLP-1 release from intestinal L-cells (82) by enhancing mitochondrial oxidative phosphorylation and calcium influx, which resulted in improved liver and pancreatic function and enhanced glucose tolerance (77;81). Furthermore, the potential contribution of increased plasma BA to improved glucose metabolism has been recently reported in humans after gastric bypass surgery (75). The investigators showed an inverse correlation of total plasma BA with glucose concentrations two hours after food intake and a positive correlation to peak GLP-1 levels (75).

Previous trials have shown that leptin modulates pancreatic beta-cell functions through direct actions (32;83) and indirectly through central neural pathways (84). Leptin receptors are present in pancreatic beta-cells (33). In physiological concentrations (1.7 to 10.0 ng/mL), leptin significantly down-regulates insulin gene expression and secretion from beta-cells in the presence of high glucose concentrations as shown in fed mice (85). Leptin specifically inhibits glucose-stimulated insulin secretion via the cAMP/protein kinase A (PKA) or phospholipase C/protein kinase C (PKC) pathways (86). Peripherally, leptin activates signalling pathways that are similar, albeit not identical, with those activated by insulin (87). Leptin may alleviate increased glucagon concentration that is often found in patients with LC, which may contribute to improved insulin sensitivity (88).

As a result of these scientific indications, we were interested to exploratively associate plasma BA and leptin concentrations with parameters of glucose metabolism in patients with LC and in situ transjugular intrahepatic portosystemic shunts (TIPS) under fasting conditions, after oral nutritional intake and during parenteral nutrition. In patients with LC, the placement of TIPS causes sustained increases in plasma BA, insulin and glucagon levels (89) without affecting fasting glucose and C-peptide concentrations or glucose tolerance. Thus, we considered this human model efficient for testing the hypothesis that BA possible relate to glucose tolerance in patients with LC.

1.5 Peptide YY in patients with liver cirrhosis

In 2002, Batterham and colleagues first reported that peripheral administration of peptide YY (PYY) reduces food intake in rodents and normal-weight humans (90;91). This anorectic action has been reproduced in rodents, in non-human primates and in humans (92). PYY is now considered an important humoural mediator for delayed gastric emptying and prolonged intestinal transit time. Furthermore, several recent studies have suggested that PYY interacts with the regulation of energy expenditure (93;94), albeit the mechanisms underlying these effects remain to be determined.

PYY is a short 36 amino acid molecule that is synthesised in response to nutritional stimulus by the L-cells of the gut mucosa. L-cells are predominantly located in the distal gastrointestinal tract (95). PYY is secreted into the circulation in proportion to meal size, similar to cholecystokinin (CKK) and GLP-1 (92;96). PYY_{3-36} is the truncated 34-amino acid form produced from PYY by cleavage of the N-terminal tyrosin-prolin residues. PYY_{3-36} is the main circulating form of the peptide (92;97) and accounts for the majority of its effects.

Anorexia, delayed gastrointestinal transit and abnormal energy expenditure are often observed in patients with decompensated LC (63;98;99), contributing to the high prevalence of malnutrition in this patient group (100). Interestingly, insertion of TIPS apparently increases appetite and voluntary food intake by an average of 30%, resulting in persistent weight gain (64).

This part of our investigations aimed at exploring whether circulating levels of PYY_{3-36} and energy metabolism are different in patients with decompensated LC without TIPS compared to patients with compensated LC and in situ TIPS and healthy controls under fasting conditions, after oral nutrition and during parenteral nutrition.

1.6 Hypotheses

In our explorative investigations we hypothesised:

Referring to 1.1 and 2.1:

- That a significant percentage of patients with quiescent IBD will be classified as being malnourished by the Subjective Global Assessment (SGA), the body mass index (BMI) and by serum albumin concentration.
- That the prevalence of malnutrition is higher in patients with CD than in patients with UC.
- That even patients with IBD classified as well-nourished differ in body composition and muscle strength from sex-, age- and BMI-matched controls in paired analyses.
- That change in the nutritional status, body composition and muscle strength correlate to intestinal and systemic inflammation, disease location as well as to present and past medication.

Referring to 1.2 and 2.2:

- That, in patients with IBD, plasma concentrations of adipokines differ significantly from those of healthy controls and that change is more prominent in patients with active disease.
- That change in the adipokine pattern predisposes patients with IBD to insulin resistance.
- That change in adipokines is associated with intestinal and systemic inflammatory markers, disease activity, disease location and the rate of relapse.

Referring to 1.3 and 2.3:

- That plasma bile acids (BA) correlate to energy expenditure in patients with liver cirrhosis (LC) but not in healthy controls.
- That this correlation can contribute to explaining hypermetabolism in patients with LC.
- That correlation between BA and energy expenditure is mainly observed during the postprandial period and not under fasting conditions.
- That the association with energy expenditure differs between BA subgroups.
- That plasma thyroid hormones respond to the BA-induced stimulation of energy expenditure.

Referring to 1.4 and 2.4:

- That both plasma bile acids (BA) and leptin are involved in the regulation of glucose metabolism in patients with hepatogenous insulin resistance due to liver cirrhosis.
- That the involvement of BA and leptin is present under fasting conditions, after oral nutritional intake and potentially during parenteral nutrition.
- That leptin is released viscerally from the intestine in patients with LC.

Referring to 1.5 and 2.5:

- That patients with decompensated LC have increased plasma peptide $YY_{3-36,}$ which might contribute to the often observed status of anorexia in this group.
- That patients with LC show normalising peptide YY_{3-36} after insertion of a transjugular intrahepatic portosystemic shunt (TIPS), which may contribute to explaining the increased appetite and voluntary food intake observed in this group.

2 Own work

The present work includes five original papers. The following questions were evaluated for patients with inflammatory bowel diseases (IBD) and for patients with liver cirrhosis (LC):

For patients with mainly clinically quiescent IBD

1) What are the nutritional status and the prevalence of malnutrition in quiescent inflammatory bowel diseases?
3) Does the disease (past and current medication, clinical course, etc.) cause persisting changes in body composition? Are these changes observed even in well-nourished patients with IBD?
4) Are changes in body composition associated with systemic or intestinal inflammatory markers (CRP, IL-6, faecal calprotectin)?
5) Is IBD associated with changes in circulating adipokines?
6) Is nutritional status or body composition associated with quality of life and muscle strength in patients with IBD?
7) Are glucose and insulin metabolism normal in patients with IBD?

For patients with mainly stable LC and in situ transjugular intrahepatic portosystemic shunt (TIPS)

1) Are BA involved in the energy metabolism of patients with LC or healthy controls? If yes, are specific subgroups responsible for the observed associations?
2) Are circulating BA associated with thyroid hormones?
3) Are circulating BA involved in the glucose metabolism of patients with LC?
4) Are plasma concentrations of the anorexigenic neuropeptide peptide YY (PYY) normal in patients with LC?

2.1 Selected micronutrient deficits and loss of body cell mass are frequent in patients with quiescent inflammatory bowel diseases

Luzia Valentini, Lennart Schaper, Carsten Buning, Susanne Hengstermann, Thomas Koernicke, Wolfgang Tillinger, Francesco William Guglielmi, Kristina Norman, Sabine Buhner, Johann Ockenga, Matthias Pirlich, Herbert Lochs. Malnutrition and impaired muscle strength in patients with Crohn's disease and ulcerative colitis in remission. Nutrition (2008) 24:694-702

This multicentre trial investigated the nutritional status of 144 patients with IBD from three European centres. Clinical remission was defined as Crohn's Disease Activity Index (CDAI) < 150 (103;104) or Colitis Activity Index < 5 (CAI) (105). For an in-depth evaluation of body composition, 47 healthy controls were recruited, each matching one specific well-nourished patient with IBD (same sex, BMI ± 0.3 kg/m², age ± 5 years); paired analysis was conducted in this subgroup.

Overall, 88.2% of the patients with IBD were well-nourished according to the Subject Global Assessment (SGA), the gold standard for the identification of malnutrition. All patients classified as well-nourished had normal serum concentrations for albumin, total protein, zinc and folate, but a significant number of patients showed low levels of selenium, magnesium, B12 and ferritin. Pair-matched analysis in well-nourished patients with IBD showed a significant shift in body composition towards higher fat mass and lower lean body mass. Changes in body composition were neither associated with location and duration of disease, current disease activity, intestinal resections nor with quality of life. However, patients with CRP concentrations above the reference range (8 to 40 mg/L) had significantly lower body cell mass (BCM) than patients with normal CRP levels, which points towards a possible role of chronic inflammation in the alterations in body composition. We concluded that selected micronutrient deficits and loss of BCM are frequent in patients with quiescent IBD and that tools for screening standard malnutrition are inadequate for detecting such deficits in this group of patients.

2.2 Pro-inflammatory adipokines are increased in patients with inflammatory bowel diseases

Luzia Valentini, Eva Katrin Wirth, Ulrich Schweizer, Susanne Hengstermann, Lennart Schaper, Thomas Koernicke, Ekkehart Dietz, Kristina Norman, Carsten Buning, Brigitte M. Winklhofer-Roob, Herbert Lochs, Johann Ockenga. Circulating adipokines and the protective effects of hyperinsulinaemia in inflammatory bowel disease. Nutrition (2009) 25:172-181

This cross-sectional trial including 128 patients with IBD and 37 healthy controls investigated circulating concentrations of adipokines (leptin, resistin, visfatin, retinol-binding protein-4, adiponectin), glucose homeostasis (fasting glucose, insulin) and inflammatory markers (CRP, IL-6 and faecal calprotectin) under overnight fasting conditions. The clinical course was followed up for 6 months for 92 patients.

The trial showed 1) that leptin in patients with IBD was similar to controls 2) that resistin was increased in active disease in patients with CD and UC and that it was correlated to disease activity scores, CRP levels and faecal calprotectin 3) that visfatin was increased in active UC without correlating to inflammatory markers 4) that RBP-4 was increased in all patient groups and positively associated with total serum fatty acids 5) that adiponectin was decreased in all patient groups independent of disease activity and that it correlated negatively to RPB-4 and body fat mass. Hyperinsulinaemia was common in patients with IBD (60%) and was negatively associated with the adiponectin concentration ($p < 0.001$); furthermore, hyperinsulinaemia proved to be an independent protective factor for 6 month maintenance of remission.

We concluded that IBD leads to largely similar alterations in circulating adipokines in patients with Crohn's disease as well as in patients with ulcerative colitis. The unexpected protective effect of hyperinsulinaemia on relapse pointing towards an adaptive mechanism requires further investigation.

2.3 Plasma bile acids are associated with energy expenditure and thyroid function

Johann Ockenga, Luzia Valentini*, Tatjana Schuetz, Franziska Wohlgemuth, Silja Glaeser, Ajmal Omar, Esmatollah Kasim, Daniel duPlessis, Karen Featherstone, Julian R. Davis, Uwe J. F. Tietge, Thomas Kroencke, Heike Biebermann, Josef Köhrle, Georg Brabant. Plasma bile acids are associated with energy expenditure and thyroid function in humans. Journal of Clinical Endocrinology and Metabolism (2012) 97(2):535-542*

***** equally contributed

Trials in mice have indicated that bile acids (BA) play an important role in increasing energy expenditure through activation of the TGR-5 / adenylate cyclase / deiodinase type 2 pathway (58).

To evaluate these findings in humans, we investigated possible correlations between energy expenditure, serum bile acids (BA) and thyroid hormones in eight patients with increased BA concentrations due to liver cirrhosis (LC) and ten healthy subjects with normal BA concentrations under baseline fasting conditions and after oral nutrition.

We found significant positive associations between BA and energy expenditure at baseline with the highest correlations to deoxycholic acid, a secondary BA generated by colonic microbiota. Postprandially, the correlation to energy expenditure resolved but, for patients with LC, postprandial changes in the thyroid-stimulating hormone (TSH) correlated significantly to the meal-stimulated BA increase. When investigating the underlying mechanisms, we predominantly found cytoplasmatical expression of TGR-5 human TSHoma cells. In vitro stimulation with BA did not substantially alter cAMP or deiodinase type 2 concentrations in TαT1 mouse thyrotroph cells.

We concluded that our data support a role of BA in human energy expenditure and in thyroid hormone control in health and disease. Possibly, BA are involved in the fine tuning of homeothermia. However, we were unable to obtain any convincing evidence for the underlying mechanism.

2.4 Plasma bile acids are associated with postprandial glucose tolerance in patients with liver cirrhosis

Luzia Valentini, Silja Glaeser, Tatjana Schuetz, Ajmal Omar, Esmatollah Kasim, Thomas Kroencke, Uwe J.F. Tietge, Herbert Lochs, Jörg-Dieter Schulzke, Georg Brabant, Johann Ockenga. Serum bile acids and leptin interact with glucose metabolism in patients with liver cirrhosis. Clinical Nutrition (2012) http://dx.doi.org/10.1016/j.clnu.2012.06.006

Recent evidence from human trials has attributed the immediately improved glucose tolerance after Roux-en-Y gastric bypass surgery to the persisting increases in circulating bile acids (BA) as a result of this surgical procedure (75).
Therefore, we investigated plasma BA and glucose metabolism in patients with liver cirrhosis (LC) with increased serum concentrations of BA and signs of hepatogenous insulin resistance. In addition, we examined a possible association with plasma leptin concentrations, because leptin was related to insulin concentrations in one of our previous clinical studies on patients with LC (106).

In total, ten cirrhotic patients and ten healthy subjects were investigated at baseline, after oral nutrition and during parenteral nutrition. At baseline, both fasting BA and fasting leptin correlated to insulin resistance according to the homeostasis model assessment (HOMA). After oral nutritional stimulus, both fasting BA and fasting leptin were independent predictors of insulin response. Postprandial glucose response was negatively associated with postprandial BA increase. During parenteral nutrition, however, only leptin did independently predict insulin response.
Our results suggest that plasma BA and leptin play a role in the glucose metabolism of patients with LC by improving postprandial glucose tolerance. The relations were only present in patients but not in healthy subjects.

2.5 Plasma peptide YY (PYY) is increased in patients with decompensated liver cirrhosis

Luzia Valentini, Tatjana Schuetz, Ajmal Omar, Silja Gläser, Esmatollah Kasim, Peter Nowotny, Thomas Kroencke, Johann Ockenga. Abnormal plasma peptide YY_{3-36} levels in patients with liver cirrhosis. Nutrition 2011; 27:880-885

Because anorexia and malnutrition are often observed in patients with decompensated liver cirrhosis (LC), we investigated fasting and postprandial peptide YY_{3-36} (PYY_{3-36}) plasma concentrations in this group of patients.

PYY_{3-36} was analysed in six patients with decompensated LC, in nine patients with compensated LC and in situ transjugular portosystemic shunts (TIPS) and in ten healthy controls under fasting conditions, after oral nutrition and during 1-hour continuous parenteral nutrition.

Under fasting conditions, plasma PYY_{3-36} concentrations were significantly higher in patients with decompensated LC than in patients with compensated LC or in healthy controls. Postprandially, PYY_{3-36} increased in patients with decompensated LC and in healthy controls, but not in patients with compensated LC. Parenteral nutrition did not significantly affect PYY_{3-36} in any of the study groups.

The authors concluded that neuroendocrine regulation of PYY_{3-36} was abnormal in patients with LC. In patients with decompensated LC, increased fasting PYY_{3-36} may contribute to anorexia. In patients with TIPS, the absent postprandial anorexigenic stimulus may play a part in the increased appetite and energy uptake previously shown in this group of patients with compensated LC (64).

3 Discussion

3.1 Summary of results and their relevance for clinical nutrition for patients with inflammatory bowel diseases

Our two prospective controlled multicentre trials including 183 patients with inactive and active inflammatory bowel diseases (IBD) (107;108) showed several alterations in the nutritional status of these patients compared to healthy controls. With regard to our hypotheses, these alterations can be summarised as follows:

First, in clinical remission, the prevalence of overt malnutrition was unexpectedly low for both Crohn's disease (CD) and ulcerative colitis (UC). Only 11.8% of patients with IBD were moderately malnourished according to the SGA, 4.9% had a BMI below 18.5 kg/m², 6.9% showed a weight loss of more than 5% and 10.4% had low albumin values. These figures are in line with later results by Bin and colleagues in inactive CD (109) showing an 18.7% prevalence of moderate malnutrition for SGA and a 6.7% prevalence of underweight according to the BMI. We additionally observed selected micronutrient deficiencies affecting up to 77% of patients (108). A further evaluation in the same study population showed decreased serum concentrations of antioxidant vitamins, provitamins and carotenoids, such as vitamin C, β-carotene, α-carotene, lutein/zeaxanthin, β-cryptoxanthin, lycopene, and total carotenoids (110). Post assessment of our patients with CD (unpublished) showed that plasma CRP levels were negatively associated with vitamin C concentration ($r = -0.278$, $p = 0.014$), and trends were seen for β-carotene ($r = -0.171$; $p = 0.061$). Moreover, faecal calprotectin, a marker of intestinal inflammation, was negatively associated with plasma concentrations of vitamin C ($r= -0.452$, $p = 0.004$) and β-carotene ($r = -0.328$, $p = 0.012$). These additional results pointed towards the substantial involvement of systemic and intestinal residual inflammatory activity in the development of nutrient deficiencies in patients with clinically quiescent IBD that cannot be detected by existing malnutrition screening tools. Therefore, the development of new screening tools that integrate inflammatory parameters are warranted for this patient group.

Second, even patients with clinically quiescent IBD classified as well-nourished significantly differed in body composition, showing a significant shift towards higher body fat mass and lower body cell mass than healthy controls with a similar body mass index (BMI). Increased visceral and mesenteric fat mass with an increased ratio of intra-abdominal fat to total abdominal fat was previously reported for patients with IBD (22-24); however, we were the first authors to show a relative increase in the subcutaneous fat mass of these patients.

Interestingly, general epidemiologic trends exist for a steadily increasing BMI and thus body fat mass in patients with IBD (111;112). We further found increased plasma concentrations of total fatty acids, saturated fatty acids and monounsaturated fatty acids but not for polyunsaturated fatty acids (107). Detailed analyses of plasma fatty acids showed that increased plasma concentrations were limited to seven particular fatty acids, namely pentadecanoic acid (C15:0), hexadecanoic acid (C16:0), octadecanoic acid (C18:0), 11-decenoic acid (C18:1n-7), 9-octadecenoic acid (C18:1n-9), tetracosanoic acid (C24:0) and *all-cis*-7,10,13,16-docosatetraenoic acid (C22:4n-6) (110). Knowledge on the plasma fatty acid pattern may help identify the underlying mechanisms for the observed changes in patients with IBD in future.

Third, in accordance with our hypothesis, muscle strength was significantly lower even in well-nourished patients with IBD than in healthy controls. Decreased muscle strength has been recently confirmed in another trial for over 70% of patients with clinically quiescent CD (109). In our patients, muscle strength was negatively associated with systemic but not with intestinal inflammation.

Fourth, in contrast to our hypothesis, changes in nutritional status or body composition were neither associated with the location and duration of the disease nor with current disease activity (CDAI, CAI) or intestinal resections. Previous prednisolone therapy with a cumulative dosage of more than 1 g affected the BCM in women but not in men. However, confirmation of this gender-specific effect by other investigations is still lacking.

Fifth, we observed several alterations in the plasma adipokines of patients with IBD that were largely similar in UC and CD but unrelated to disease duration, localisation and actual or previous medication. We showed associations with parameters representing the lipid compartment also for adipokines. RBP-4, an adipokine closely associated with visceral fat mass (51), was increased and positively associated with total plasma fatty acid concentration (107). The plasma concentration of adiponectin, an anti-inflammatory adipokine (43), was negatively associated with subcutaneous fat mass (107).

Sixth, hyperinsulinemia was observed in about 60% of our patients. Interestingly, plasma insulin concentration was negatively correlated to adiponectin levels, and this change was predictive for the 6 month maintenance of remission for our patients. However, this result should also be interpreted cautiously because confirmation by other trials is still lacking. Nonetheless, both the significantly increased fasting insulin concentration and higher HOMA values have been verified in a more recent investigation in patients with IBD (113).

Furthermore, in the same trial, the investigators found increased carotid intima-media thickness and carotid artery stiffness and thus an increased risk of early atherosclerosis in patients with IBD (113).

In conclusion, for patients with quiescent IBD, our unexpected main result was the consistent change ─ mostly an increase ─ of values linked to the lipid compartment of the human body. These increased values especially may seem paradoxical at first, but new evidence suggests that adipose tissue is closely involved in the pathogenesis of IBD (114). For example, fatty acids may influence immune function through a variety of mechanisms, and many of these are associated with changes in the fatty acid composition of immune cell membranes (115). Fatty acids can modify membrane fluidity, lipid raft formations and phopholipid-based cell signalling, resulting in altered gene expression and changed pattern of lipid mediator production (115). Furthermore, a recent publication has suggested that adipokines derived from the mesenteric fat of patients with CD shape the local macrophage compartment and possibly exert protective effects (31). This finding is even more interesting from an evolutionary point of view, because immune cells and adipose tissue have evolved from common ancestral structures (116). The rational for this closely linked coordination of metabolic and immune responses includes the evolutionary advantage of locally centred organisation and redistribution of energy resources during the mounting of immune or inflammatory responses (116). During a metabolic-inflammatory response of the organism, the neuroendocrine signals of the adipokine system may modulate the metabolic and immunological activities for the benefit of the individual (117).

In conclusion, compromised anti-oxidative status and changes in both fat mass and lipid parameters strongly suggest anti-inflammatory nutritional strategies in patients with clinically quiescent IBD. So far, the provision of n-3 fatty acids has been the only anti-inflammatory strategy intensively investigated in patients with IBD.

3.1.1 n-3 fatty acids as an anti-inflammatory nutritional strategy in patients with inflammatory bowel diseases

Omega-3 (ω-3 or n-3) fatty acids belong to the group of dietary-derived polyunsaturated fatty acids (PUFAs). The term n-3 refers to the position of the double bond nearest the methyl carbon end of the fatty acid. The simplest n-3 fatty acid is α-linolenic acid (18:3n-3) (118). α-Linolenic acid is produced in plants that can be converted into small amounts of eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in humans. EPA and DHA are the actual bioactives

responsible for the anti-inflammatory effects of n-3 fatty acids. In dietary sources, significant quantities of EPA, DPA and DHA are found in seafood, particularly in oily fishes (119). A single lean fish meal (e.g. one serving of cod) can provide about 0.2 to 0.3 g of marine n-3 PUFAs, whereas a single oily fish meal (e.g. one serving of salmon or mackerel) can provide 1.5 to 3.0 g of these fatty acids (118). Fish oil supplements typically contain 30% of n-3 PUFAs (EPA, DHA). Thus, 1 g of fish oil capsule will provide about 0.3 g of EPA and DHA (118).

EPA and DHA seem to decrease chemotaxis of neutrophils and monocytes by unclear mechanisms and with suggested near-maximum inhibition at 1.3 g EPA+DHA/day (120). Supplementation of 1.5 g/d EPA+DHA in healthy humans resulted in lower expression of the intercellular adhesion molecule (ICAM-1) on blood monocytes stimulated ex vivo with interferon-γ (121). 1.8 g/d EPA+DHA administered to patients with peripheral vascular disease decreased the adhesive interaction of monocytes with the endothelial monolayer in cultures (122). In elderly people, 1.1 g/d EPA+DHA reduced the serum levels of the soluble vascular cell adhesion molecule (VCAM-1) (123), and 1.8 g/d EPA decreased soluble ICAM-1 and soluble VCAM-1 (124) in patients with metabolic syndrome. In addition, EPA or DHA reduced the production of arachidonic acid-derived pro-inflammatory eicosanoids, such as prostaglandin E_2 (PGE_2) (118). EPA and DHA supplementation in healthy humans decreased the production of TNF-α, IL-1β and IL-6 by endotoxin-stimulated monocytes in several trials, but not all clinical studies confirmed the effect and dosages of at least 2 g EPA+DHA seem to be required to induce this effect (118). With regard to reduced T-cell proliferation, human trials with EPA+DHA supplementation are not consistent (118). In summary, these trials have shown that, in humans, a dosage of 2 g/d and more of marine n-3 PUFAs exert a range of anti-inflammatory effects by multiple mechanisms (125).

The mechanisms involved include an increased net incorporation of EPA and DHA in phopholipid cell membranes ─ which are involved in inflammation ─, resulting in a parallel decrease in incorporated arachidonic acid (125). This change peaks within a few weeks of supplementation and occurs in a dose-response manner (126), altering the availability of substrates for the synthesis of eicosanoids, endocannabinoids, resolvins and protectin (125). Further mechanisms may involve “lipid rafts”, i.e. essential structures formed by the movement of receptors, accessory proteins and enzymes within the plane of the cell membrane (125). Cell and animal models have shown that marine n-3 PUFAs modify raft formation in T-cells in a way that impairs the intracellular signalling mechanism in these cells (127). This finding is consistent with the decreased T-cell reactivity observed after EPA+DHA exposure (118). Another mechanism by which marine n-3 PUFAs are suggested to exert their anti-inflammatory effects is the inhibited activation of the transcription factor nuclear

factor kappa B (NFκB) in response to exogenous inflammatory stimuli (118). DHA and EPA binding to G-protein coupled receptor (GPR) 120 seem to be involved in the inhibition of NFκB activation (128). Furthermore, evidence exists that the peroxisome proliferator-activated receptor (PPAR)-γ can be activated by n-3 PUFA and that these effects are linked to the decreased production of TNF-α and IL-6 upon endotoxin stimulation (129).

Several randomised controlled trials of n-3 PUFA supplementation in patients with IBD have reported clinical benefits, such as improved clinical scores, improved gut mucosal histologies, improved sigmoidoscopic scores, lower rates of relapse and decreased use of corticosteroids (130). The dose of marine n-3 PUFA used in these trials was high, i.e. between 2.5/d and 6 g/d, averaging about 4 g/d (115;131;132). The authors of a systemic review identified 13 trials on fish oil supplementation in patients with IBD (133). The investigators concluded that the available data were insufficient to conduct a meta-analysis except for relapse in UC, and here not benefit of supplementation was observed (133). More recent meta-analyses considering maintenance of remission in patients with CD and UC identified only marginal effects if any (134-136). Thus, despite generally good scientific reprocessing and some favourable human trials, at best only weak evidence exists that marine n-3 PUFAs are clinically beneficial in human IBD (118;137).

Overall, the available data are insufficient to support the use of n-3 PUFA as an anti-inflammatory nutritional strategy in active or inactive IBD (137). Negative results are rather consistent in trials assessing the use of n-3 PUFA to maintain disease remission, particularly in CU and, to a lesser extent, in CD (137-139).

3.1.2 Potential new nutritional strategies in patients with IBD: The example of polyphenols

Polyphenols are phytochemicals that are abundant in food derived from plants (140;141). Polyphenols belong to an ill-defined group of about 8000 secondary plant metabolites, for which no deficiency state has yet been described, thus denying these phytochemicals the title of essential micronutrients (141). These compounds are structurally characterised by the presence of one or more phenol rings and two or more hydroxyl groups that are directly linked to the aromatic rings. In plants, polyphenols have many functions, for example, colouring, protection from UV radiation, antioxidant protection from free radicals generated during photosynthesis and many more (142). The average daily intake of polyphenols in humans is approximately 1 g/d (143). This intake can be expected to be significantly lower in

patients with IBD, because they eat less fruits and vegetables (108), mainly to avoid gastrointestinal symptoms (144).
The intestinal absorption of polyphenols depends on their chemical structure and is very low, for example, for polyphenols belonging to the groups of soluble tannins (ellagitannins) (145), condensed tannins (proanthocyanidins) (146) and anthocyanins (147). These polyphenols are promising candidates for positively influencing intestinal inflammation because they are supposed to directly exert their effects in the intestine.

At the molecular and cellular level, polyphenols can act as antioxidant, anti-apoptotic, anti-carcinogenic, anti-inflammatory, anti-angiogenic and anti-cell proliferator agents (140). The general anti-inflammatory effects of polyphenols have been discussed in a number of reviews (e.g. (148)). Recently, dietary polyphenols have been suggested as a supportive treatment in patients with IBD (141;149), but so far only 12 trials have examined their effects on intestinal inflammation in vivo and in vitro (140). Polyphenols are assumed to be able to play an anti-inflammatory role via the modulation of intracellular signalling cascades in the intestinal cells with dosages achievable by fruit, vegetable and pulses intake (140). But further investigations are required in this respect.

Other functional nutritional compounds may also be promising for the treatment of IBD. A recent review has suggested that the manipulation of intestinal microbiota by dietary compounds may be a possible future nutritional strategy to influence intestinal inflammation (150). One such strategy could be the use of fermentable fibre (soluble, insoluble) and resistant starch to generate short chain fatty acids (SCFA) via intestinal microbiota. Butyrate (C4), propionate (C3) and acetate (C2) are the three main short chain fatty acids (SCFA) produced by anaerobic bacterial fermentation in the colon. The main bacteria genus producing butyrate is the Clostridium cluster IV (151), which are gram-positive bacteria of the phylum of Firmicutes. Although butyric acid in small amounts is a natural component of food such as butter or cheese, oral intake in relevant amounts is impeded by the strong, rancid and vomit-like odour of the substance. US anti-abortion extremists even used butyric acid attacks as a weapon against nearly 100 abortion facilities with the goal to disrupt services and close the clinics (152). Butyrate generated by intestinal microbiota, however, has a high potential to benefit patients with IBD because gastrointestinal epithelia use butyrate to adapt proliferation and apoptosis (153). Butyrate further inhibits release of the tumour necrosis factor α (TNF-α), IL-13 and histone deacetylase, thereby contributing to restoring the intestinal barrier of patients with IBD (153). SCFA are also recognized as potential mediators of intestinal immune function (154). The evidence on iron, calcium and Vitamin D has been recently reviewed (6), but these constituents represent only a small part of human nutrition.

Up to now, the focus in dietary recommendations for patients with IBD has been mostly set on food that potentially triggers symptoms or disease. However, future novel nutritional strategies must balance those factors. For example, the quantity of pre-illness intake of mono- and disaccharides, sweeteners, intake of total fat, monounsaturated fatty acids and PUFA as well as diets low in fruits and vegetables seem to be positively associated with the incidence of CD and UC (155-157). A prospective cohort study by Jowett et al (158) on patients with quiescent UC found that meat, protein and alcohol increase the likelihood of a relapse. A recent report from New Zealand has shown that chillies, cola, curries, corn, cream, beer and red wine commonly cause increased abdominal symptoms, but no food has been found to be universally detrimental (144).

3.2 Summary of results and their relevance for clinical nutrition for patients with liver cirrhosis

We provided preliminary evidence that BA are involved in the regulation of energy expenditure (159) and in glucose metabolism (160). Most associations were identified in patients with about 10-fold increased plasma BA concentrations due to liver cirrhosis (LC), although baseline correlation to energy expenditure was also seen in healthy subjects (159).

With regard to energy expenditure, we also surprisingly observed a correlation to BA in healthy controls with normal BA concentrations, which was contrary to our hypothesis. Nevertheless, clinical practice indicates possible associations between BA and energy expenditure in humans with altered BA metabolism but not necessarily with increased plasma BA concentrations. For example, weight gain often occurs after cholecystectomy. Houghton et al reported (161) a mean weight gain of 4.6% in men and of 3.3% in women within 6 months after cholecystectomy, although one third of the patients were already overweight at the time of surgery. Ali and colleagues (162) showed that the BMI of cholecystectomised patients increased by 1.8 kg/m² within three years after cholecystectomy in contrast to patients after non-biliary surgery. This weight gain could be caused by altered plasma BA concentrations based on changed enterohepatic cycling of BA, but this suggestion is speculative.

One common treatment of patients with primary biliary cirrhosis is BA supplementation with ursodeoxycholic acid (163), which may result in unwanted weight gain (164;165), as recently confirmed by a Cochrane meta-analysis of 11 trials (166). The underlying mechanism of such

weight gain is unclear and requires further investigation but may be linked to the manipulation of energy expenditure induced by an altered serum BA composition.
In our trial (159), two out of eight patients with liver cirrhosis were hypermetabolic, and this 25% rate fit well into the prevalence rate of previous studies (63;65;106). However, these two patients had neither the highest BA level nor did they behave differently in regard to BA to energy expenditure relations compared to normometabolic patients or controls. Therefore, we had to refute our initial hypothesis of BA being the underlying cause for hypermetabolism and concentrated on the unexpected multiple correlations between BAs and energy expenditure found in both patients and controls.

With regard to glucose metabolism in patients with LC, we could confirm our initial hypothesis on the substantial involvement of BA and leptin in hepatogenous insulin resistance. Still, our evidence stems from a small sample and requires confirmation by further investigations.

Supposed that our observations can be confirmed in future, a recently published investigation might be of special interest for future nutritional strategies regarding BA (76). Steiner and colleagues (76) showed that plasma levels of secondary or unconjugated BA (both derived from intestinal microbiota) increase substantially during the night hours; the main BA converting bacterial groups were lactobacillus, bifidobacteria, enterobacter, bacteroides and clostridium (151). At least strains of lactobacilli and bifidobacteriacae are commonly used as probiotics in different foods. Maybe probiotics can be used in future to manipulate the nightly BA harvest into the systemic circulation.

The results of our investigations of patients with LC (159;160) envision a potential of BA in modulating energy expenditure and glucose metabolism but not only in this patient group. We also observed a potential relevance of increased PYY plasma concentration in the development of anorexia in LC (167). But the outcome of all three LC investigations (159;160;167) is preliminary and needs confirmation by forthcoming studies. Prior to that, the evaluation of BA as possible target for dietary manipulations does not seem reasonable.

4 Conclusion

The presented trials dealt with five selected aspects of pathophysiology and metabolism in patients with IBD and in patients with LC. These experimentally oriented investigations shared one common implication; they all provided first concrete information for the development of evidence-based dietary concepts in clinical nutrition, an area that is largely under-investigated so far.

The implications of our trials for future concepts in clinical nutrition include the acknowledgement of the effects of low-grade inflammation on nutritional status in chronic disease, even in the absence of weight loss. Up to now recent weight loss has been one of the main operative criteria for the definition of disease-related malnutrition (168). Interestingly, meanwhile both the European (ESPEN) as well as the American Society for Clinical Nutrition (ASPEN) endorse new aetiology-based definitions, in which malnutrition in the presence of low- to medium-grade inflammation constitute an own category (169-170). However, the operative criteria for its detection are not yet defined. Therefore, the next steps are defining those criteria and developing malnutrition screening tools that can detect chronic low-grade inflammation-induced malnutrition, as was present in our patients with IBD in remission, where existing standard malnutrition screening tools failed to detect malnutrition adequately.

Another implication resulting from our trials is the relevance of developing novel evidence-based anti-inflammatory nutritional regimens. These regimens should preferably include the "normal" diet as a whole, and not target at one specific nutritional supplement, because the effects of a single nutritional supplement can easily be modified by bioactive compounds present in normal nutrition. Such interaction might have contributed to the discouraging results for n-3 fatty acids in IBD patients (137-139).

As indicated earlier, existing dietary recommendations in clinical nutrition are rarely evidence-based.
In acute disease, the development of evidence-based dietary concepts is relatively simple. Nutritional therapy to support healing (in contrast to nutritional support to treat malnutrition) is a clinically non-mandatory adjunct therapy. Such a therapy may be scientifically tested either complementarily to medical treatment or, in some cases, instead of a medical drug. Adherence to the principles of evidence-based medicine via implementation of randomised controlled trials is possible, albeit not always ethically feasible, particularly in case of malnourished patients. To prove the effects of nutrition, true endpoints, such as disease

recovery or disease remission, may be used. Also advantageous in acute disease is the restricted time needed to follow a specialised nutritional therapy, because the possible interference of dietary restrictions with social life will be of limited duration. One established example is the administration of total enteral nutrition for inducing remission in Crohn's disease (7) in children that has proven to be equally effective as a steroid therapy but with fewer side effects, such as stunted growth, weight gain, acne, hirsutism and striae (102).

More difficult is the development of evidence-based dietary recommendations for patients with none-acute chronic diseases to alleviate disease progression or to maintain disease remission. These recommendations aim at long-term nutrition and thus have to consider the social implications of dietary restrictions. It is pivotal that future dietary recommendations for non-acute disease are not only scientifically tested but also easy to integrate into the professional and personal life of patients. Special foods (gluten free, lactose free, rare food) or the special preparation of food should be kept at a minimum, if possible. Recommendations should result in a personalised flexible composition of the diet to fulfil the hedonistic demands of a person. So far, dietary recommendations have mainly aimed at food intolerances and food allergies, but this approach is meant to go further than that by adding a healing or a disease maintenance effect. As mentioned before, such recommendations should preferably not target at just one nutritional supplement but the effects of food groups or diets as a whole. As a results of our investigations, we discussed that anti-inflammatory nutritional strategies directly targeted at intestinal inflammation can be beneficial for IBD patients in future. Polyphenols in fruits and vegetables and short chain fatty acids derived from dietary fibre are promising bioactive compounds in common foods to achieve these effects.

This evidence-based dietary approach is much more difficult to accomplish, as it necessitates, among others, detailed evidence-based knowledge of the effects of single bioactive compounds in common foods and how they behave in the natural food matrix and during food processing (101). Additional factors that need to be identified are the dosage to reach such effects, the interaction with other dietary bioactives and the natural variation of bioactive compounds, particularly in plant species.

Further in-depth knowledge on nutrition physiology in health and disease is required to identify new targets and novel biomarkers of exposure and effect. This was another implication for future nutritional concepts in clinical nutrition resulting from our studies. We showed that considering fat mass and its endocrine capacity can be relevant for future nutritional strategies in patients with IBD. Surprisingly, some of our observations appeared to

be disease protective rather than pathogenetic. This finding is even more interesting because, from an evolutionary standpoint, immune cells and adipose tissue have evolved from common ancestral structures.
We also identified plasma bile acids (BA) as potential modulators of energy expenditure and glucose homeostasis in humans. The results can be relevant for future nutritional strategies, because micelle formation and re-absorption of BA in the small intestine might be manipulated by dietary compounds, and the colonic harvesting of secondary BA can possibly be manipulated by dietary probiotics or prebiotics.

In summary, concepts for evidence-based clinical nutrition are still in their very early stages.
One of the first steps is to accumulate evidence on physiologic associations between disease and nutritional biomarkers as well as to investigate and identify possible future targets for dietary interventions, which was done exemplarily on selected issues in the presented investigations.

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7 Eidesstattliche Erklärung

ERKLÄRUNG

§ 4 Abs 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
- die vorgelegte Habilitationsschrift ohne fremde Hilfe verfasst, die beschriebenen Ergebnisse selbst gewonnen sowie die verwendeten Hilfsmittel, die Zusammenarbeit mit anderen Wissenschaftlern/Wissenschaftlerinnen und mit technischen Hilfskräften sowie die verwendete Literatur vollständig in der Habilitationsschrift angegeben wurden,
- mir die geltende Habilitationsordnung bekannt ist.

14.09.2012

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