Institut für Ernährungs- und Lebensmittelwissenschaften (IEL)
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Probiotics as a novel approach to modulate incretins, insulin secretion and risk factors of type 2 diabetes and complications

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Summary

***Background and Aim*:** Ingestion of probiotics can modify gut microbiota and alter insulin resistance and diabetes development in rodents. We hypothesized that daily intake of *Lactobacillus (L.) reuteri* increases insulin sensitivity by changing cytokine release and insulin secretion via modulation of glucagon-like peptide (GLP-1, GLP-2) release.

Material and Methods: A prospective, double-blind, randomized trial was performed in 21 glucose tolerant humans (10 obese; age 51±2 years, BMI 36.0±4.8 kg/m^2; 11 lean; 49±4 years, BMI 23.6±1.9 kg/m^2). Participants ingested 10^{10} *L. reuteri* or placebo b.i.d. over 4 weeks. Oral glucose tolerance and isoglycemic glucose infusion tests were used to assess GLP-1, GLP-2 and C-peptide secretion, hyperinsulinemic-euglycemic clamps with [6,6-$^2H_2$]glucose to measure peripheral insulin sensitivity (M-value) and endogenous glucose production (EGP). Muscle and hepatic lipid contents were measured by ^{1}H magnetic resonance spectroscopy. Immune status was assessed by measuring systemic cytokines, high-sensitive C-reactive protein (hsCRP) and lipopolysaccharide (LPS) concentrations.

***Results*:** Intervention did not affect body mass, ectopic fat content and circulating cytokines. M was 37% lower ($p<0.01$) in obese than in lean volunteers, but both M-value and EGP did not change upon *L. reuteri* treatment. However, administration of *L. reuteri* increased glucose-stimulated insulin and C-peptide secretion by 49% ($p<0.05$) and 55% ($p<0.05$), respectively. Moreover, administration of *L. reuteri* improved the glucose-stimulated GLP-1 and GLP-2 release by 76% and 43%, respectively, compared to placebo ($p<0.05$).

Conclusions: Enrichment of gut microbiota with *L. reuteri* increased incretin-mediated insulin and C-peptide release, without effects on insulin sensitivity in glucose tolerant human subjects. These results suggest that modifying the microbiome could increase insulin secretion and thereby serve as a novel therapeutic tool for the treatment of type 2 diabetes. However, further studies are needed to address this issue in this emerging research field.

Zusammenfassung

Hintergrund: Die Einnahme von Probiotika führt bei Nagetieren zu einer Modifizierung der Darmflora und nachfolgend zu einer Veränderung der Insulinresistenz sowie verzögerten Entwicklung eines Diabetes mellitus. Unsere Hypothese lautet, dass es durch die tägliche orale Einnahme von *Lactobacillus (L.) reuteri* über 4 Wochen und die sich daraus ergebende Veränderung der menschlichen Darmflora zu einer gesteigerten Sekretion der Darmhormone GLP-1, GLP-2, sowie einer verbesserten Insulinsensitivität und -Freisetzung kommt.

Material und Methoden: Eine prospektive, doppelblinde, randomisierte, zweiarmige Studie mit 21 Probanden mit normaler Glukosetoleranz (10 adipöse Personen, Alter 51±2 Jahre, BMI 36,0±4,8 kg/m^2, 11 schlanke Personen, Alter 49±4 Jahre, BMI 23,6±1,9 kg/m^2) wurde durchgeführt. Die Probanden nahmen konstitutiv zweimal täglich 10^{10} *L. reuteri* oder Plazebo über 4 Wochen ein. Ein isoglykämischer i.v. Glukoseinfusionstest analog zu den Blutzuckerspiegeln des vorausgegangen oralen Glukosetoleranztests wurde durchgeführt, um den Inkretineffekt sowie die Insulinsekretion zu untersuchen. Zur Messung der peripheren (M-Wert) und hepatischen Insulinsensitivität (endogene Glucose-produktion, EGP) wurde ein euglykämischer-hyperinsulinämischer Clamp unter Einsatz eines nicht-radioaktiven Tracer (6,6[$^2H_2$]Glukose) durchgeführt. Ektope Fetteinlagerungen in Muskel und Leber wurden mittels ^{1}H Magnetresonanzspektroskopie (MRS) gemessen. Der systemische Immunstatus wurde anhand systemischer Spiegel von Zytokinen, hoch sensitivem C-reaktivem Protein (hsCRP) und Lipopolysaccarid (LPS) erfasst.

Ergebnisse: Die 4-wöchige Einnahme von *L. reuteri* hatte keinen messbaren Einfluss auf Körpergewicht und Körperfettanteil. Der M-Wert von adipösen Probanden war 37% niedriger ($p<0,01$) verglichen mit dem der schlanken Teilnehmer, jedoch blieben M-Wert und EGP unter der Intervention mit *L. reuteri* oder Plazebo unverändert. Die Einnahme von *L. reuteri* führte jedoch zu einer Glucose-stimulierten Erhöhung der Insulin- (49%, $p<0,05$) und C-Peptid- Sekretion (55%, $p<0,05$). Desweiteren führte die Einnahme von *L. reuteri* zu einer Erhöhung von Glucose-stimulierten GLP-1 und GLP-2 um 76% bzw. 43% ($p<0,05$) verglichen zur Plazebo-Gruppe.

Zusammenfassung: Die tägliche Einnahme von *L. reuteri* über 4 Wochen erhöht Inkretin-vermittelt die Insulin- und C-Peptid-Sekretion, ohne jedoch die Insulinsensitivität der glukosetoleranten Probanden zu beeinflussen. Das lässt vermuten, dass die probiotische Modulation der Darmflora die Insulinsekretion verbessert und somit als therapeutischer Ansatz bei Personen mit Diabetes Anwendung finden könnte. Im Hinblick auf die aktuelle und durchaus kontroverse Informationslage in diesem Forschungsgebiet bedarf es weiterer Studien, um diesen Effekt von Probiotika noch genauer zu untersuchen.

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Abbreviations

APE	atom percent enrichment
AUC	area under curve
BMI	body mass index
CFU	colony forming unit
CRP	C-reactive protein
DAG	diacylglycerol
DI	disposition index
DDZ	german diabetes center
DPP4	dipeptidyl peptidase 4
EDTA	ethylendiamintetraacetat
FFA	free fatty acids
GC-MC	gas chromatography-mass spectrometry
GIP	gastric inhibitory polypeptide or glucose-dependent insulinotropic peptide
GLP-1	glucagon-like peptide-1
GLP-2	glucagon-like peptide-2
GOT	glutamic oxaloacetic transaminase
GPR	G-protein-coupled receptor
GPT	glutamic pyruvate transaminase
HCL	hepatocellular lipids
hsCRP	high-sensitive C-reactive protein
IcCE	individual calibration control evaluation
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IGF-1	insulin-like growth factor-1
IMCL	intramyocellular lipids
IQR	interquartile range
IRS-1 /-2	insulin-receptor substrate
ISR	insulin secretion
i.v.	intra venous
ivGTT	intra venous glucose tolerance test
LAL	limulus amebocyte lysate
LBP	lipopolysaccharide-binding protein
LCFA	long-chain fatty acids
LPS	lipopolysaccharide
MRI	magnetic resonance imaging

MRS	magnetic resonance spectroscopy
NAFLD	non-alcoholic fatty liver disease
NGT	normal-glucose tolerance
OGIS	oral glucose sensitivity index
OGTT	oral glucose tolerance test
PCR	polymerase chain reaction
PI3K	phosphatidylinositol 3-kinase
PKC	protein kinases C
REE	resting energy expenditure
ROS	reactive oxygen species
RQ	respiratory quotient
RT	room temperature
SCFA	short-chain fatty acid
SD	standard deviation
SEM	standard error of the mean
TG	triglycerides
TLR	toll-like receptor
T2D	type 2 Diabetes
VCO_2	carbon dioxide production
VO_2	oxygen consumption

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Introduction

The role of insulin resistance and secretion in type 2 diabetes

In 2010 about 285 million people were suffering from diabetes and this number is expected to increase to more than 550 million by the year 2030 (IDF, 2011; Shaw et al., 2010; Nolan et al., 2011). Type 2 diabetes (T2D), formerly known as non-insulin dependent diabetes mellitus (NIDDM), is the most prevalent form and accounts for about 90% of diabetes cases (ADA, 2010). The prevalence of T2D is assumed to increase due to population growth, aging and urbanization. It is expected that diabetes incidence, which already reached epidemic dimensions, will continue to increase (Chan et al., 2009; Roglic et al., 2005; Wild et al., 2004).

T2D results from decreased insulin sensitivity in combination with insufficient insulin secretion. When individuals are insulin-resistant and have lost approximately 65% of their β-cell function, T2D becomes overt (Meier et al., 2012; Abdul-Ghani et al., 2006a; Abdul-Ghani et al., 2010; Abdul-Ghani et al., 2006b; Ferrannini et al., 2005; Defronzo, 2009; Defronzo, 2004) (**figure 1**). Moreover, T2D is associated with reduced incretin concentrations as well as incretin effects (Toft-Nielsen et al., 2001; Vilsboll et al., 2001; Meier et al., 2001; Nauck et al., 1986), resulting in an impaired insulin secretion in response to glucose. In particular, the first-phase of insulin secretion (ISR) is diminished in T2D, indicating the important role of incretins as amplifiers of first-phase ISR (Woerle et al., 2012).

The recent increase in the global incidence of T2D, which is observed in Western countries and developing nations, suggests that most cases of this disease are caused by changes in environmental factors. Major risk factors for T2D such as overnutrition and low dietary fibre involve the gut and have been found to be associated with increased insulin resistance, decreased glucose tolerance and local or systemic low-grade inflammation (Kolb and Mandrup-Poulsen, 2010). Obesity has also been shown to associate with altered gut microbiota (Ley et al., 2005; Turnbaugh et al., 2006) which differs in patients with T2D compared to non-diabetic subjects (Larsen et al., 2010). In a metagenome-wide association study of gut microbiota it has be shown that patients with type 2 diabetes were characterized by a moderate degree of gut microbial dysbiosis, accompanied by increased abundance of opportunistic pathogenic bacteria (Qin et al., 2012).

Peripheral and hepatic insulin resistance

Insulin resistance is described as the fundamental failure to respond appropriately to insulin. Insulin resistance mainly affects the target tissues of insulin, particularly skeletal

muscle and liver, but also adipose tissue and brain (Szendroedi et al., 2012; Szendroedi et al., 2011; Bonnet et al., 2011; Harford et al., 2011; Ruan and Lodish, 2003; Ferrannini et al., 2005; Abdul-Ghani et al., 2006b; Banks et al., 2012).

Currently, it is under debate whether the peripheral (muscle) or the hepatic insulin resistance occurs first. Skeletal muscle is mainly responsible for whole-body insulin resistance, in terms of dysfunction of cellular mechanism to respond appropriately to insulin. Skeletal muscle insulin resistance and the resulting reduction of peripheral glucose uptake seems to develop early, as shown by studies in young lean individuals with muscle-specific insulin resistance (Petersen et al., 2007). As a consequence, glucose is redirected to the liver, which increases de-novo-lipogenesis with consecutive impairment of hepatic energy metabolism (Samuel and Shulman, 2012; Szendroedi et al., 2011; Defronzo and Tripathy, 2009).

However, it has also been suggested that hepatic insulin resistance is the primary event initiating to the development of diabetes. Previously, it has been revealed that disruption of hepatic insulin signaling results in fasting and postprandial hyperglycemia and the subsequent development of peripheral insulin resistance (Michael et al., 2000; Takamura et al., 2012).

Metabolic and environmental factors affecting insulin resistance

The link between elevated lipid levels and insulin resistance is widely accepted. Increased availability of free fatty acids (FFA) and subsequent ectopic intracellular lipid accumulation may trigger the development of insulin resistance. Particularly, an increased intracellular lipid contents in skeletal muscle and liver has been related to insulin resistance (Krssak et al., 1999; Szendroedi and Roden, 2009) (**figure 1**). It was postulated that, in muscle and liver, the intracellular accumulation of lipids and diacylglycerol (DAG) triggers the activation of novel protein kinases Cs (PKCs) with subsequent impairment of insulin signalling. For example, insulin-receptor substrate (IRS) 1-associated phosphatidylinositol 3-kinase (PI3K) activity is reduced in the muscles of individuals after a lipid infusion. In addition, in these individuals the insulin action in the liver, which has some similarities with the insulin action in muscle, is associated with defects in insulin signalling in the state of hepatic steatosis. Increased liver lipid content further impairs the ability of insulin to regulate gluconeogenesis and activate glycogen synthesis (Samuel et al., 2010; Samuel and Shulman, 2012).

Several other aspects including genetic factors, have been described to contribute to alterations of insulin resisitance, (Meigs et al., 2000; Pierce et al., 1995; Kaprio et al., 1992; Poulsen et al., 1999; Pratley, 1998; Herder and Roden, 2011; Sladek et al., 2007; Hemminki et al., 2010; Poulsen et al., 2009; Tattersal and Fajans, 1975). It has been demonstrated that first degree relatives of type 2 diabetic subjects have a higher risk to develop insulin resistance and subsequent type 2 diabetes (Axelsen et al., 1999; Groop et al., 1996; Stumvoll et al., 2005; Pratipanawatr et al., 2001)

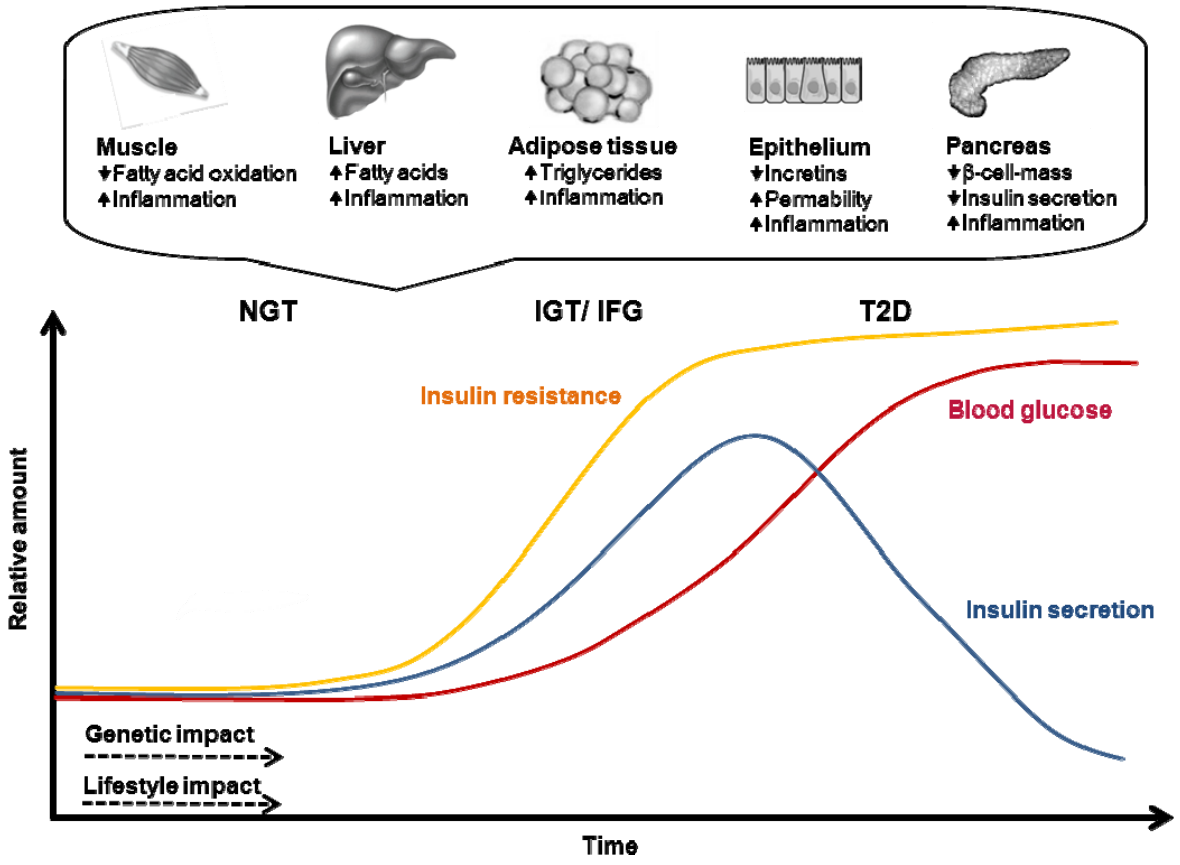

Fig. 1 The current paradigm of the development of type 2 diabetes
Type 2 diabetes (T2D) is a severe metabolic disorder characterized by a combination of insulin resistance and impaired insulin secretion. The development of T2D involves genetic, environmental, and lifestyle factors. Insulin resistance occurs in different tissues, mainly in muscle and liver and is detectable long before the onset of T2D. Temporarily, insulin resistance can be compensated by increased insulin secretion. When β-cells can no longer compensate for the insulin resistance a hyperinsulinemic state develops and T2D becomes manifest. Normal glucose tolerance (NGT), impaired glucose tolerance (IGT), impaired fasting glucose (IFG). For further details see main text. Adapted from (Kendall et al., 2009).

Adiposity, inflammation, and lifestyle factors including dietary habits and physical activity also contribute to the development of insulin resistance and subsequently to type 2 diabetes.

Adiposity contributes to T2D development in two aspects. On one hand insulin resistance of adipose tissue insulin resistance and elevated lipolysis increase levels of circulating FFA and thereby contribute to the development of insulin resistance as described above (Kashyap and Defronzo, 2007; Szendroedi and Roden, 2009). On the other hand, adipose tissue is an endocrine organ which secretes hormones such as adiponectin and leptin as well as cytokines and chemokines (Rosen and Spiegelman, 2006; Fantuzzi, 2005; Wellen and Hotamisligil, 2005; Samaras et al., 2010; Guilherme et al., 2008) contributing to the subclinical inflammation, associated with the development of T2D (Spranger et al., 2003; Herder et al., 2005b; Herder et al., 2009a; Carstensen et al., 2010; Meier et al., 2002; Pradhan et al., 2001).

Dietary habits of the western lifestyle, such as consumption of fast food, are associated with insulin resistance (Pereira et al., 2005). In addition, high-fat diet (Badin et al., 2013; Atkinson et al., 2013; Lottenberg et al., 2012) and reduced consumption of dietary fiber, especially cereals and / or carbohydrates with low glycaemic index are associated with insulin resistance (Breneman and Tucker, 2012; Brockman et al., 2012; Cloetens et al., 2012; Pal and Radavelli-Bagatini, 2012; Parnell and Reimer, 2012; Roelofsen et al., 2010; Robertson et al., 2003; Pereira et al., 2002; Weickert et al., 2006a). The consumption of dietary fiber has been suspected beneficial in several aspects. It can increase the production of short-chain fatty acids (SCFA) in the colon by increased bacterial fermentation of indigestible dietary fibers which in turn may improve lipid homeostasis and reduce hepatic glucose output (Galisteo et al., 2008). These metabolic alterations are mediated by the secretion of gastrointestinal hormones like ghrelin, peptide YY (PYY), and glucose-dependent insulinotropic peptide (GIP) with subsequent alteration of satiety (Weickert et al., 2006b; Heini et al., 1998; Weickert et al., 2005; Robertson et al., 2003; Schenk et al., 2003; Qi et al., 2005). Hence, the mechanisms through which these different diets promote the progression to insulin resistance and consecutively towards a pre-diabetic state involve a complex physiology of glucose homeostasis (Thomas and Pfeiffer, 2012).

Physical activity also seems to have a strong impact on glucose homeostasis. As mentioned above, there is an association of altered skeletal muscle mitochondrial function and changes in hepatic glucose and lipid metabolism subsequent to altered insulin sensitivity. Several studies described that physical activity protects from insulin resistance and T2D, by reversing the negative effects of insulin resistance on skeletal muscle and liver (Rabol et al., 2011; Phielix et al., 2012; Phielix et al., 2011; Boushel et al., 2007; Mogensen et al., 2007; Kelley et al., 2002; Dela et al., 1995; Manson et al., 1991; King et al., 1987).

Clinical manifestation of insulin resistance

Insulin resistance is detectable several years before the diagnosis of diabetes and is initially compensated by increased insulin secretion (Tabak et al., 2009; Ferrannini et al., 2005). T2D manifests when high insulin demand resulting from insulin resistance is no longer compensated by the β-cells (Ferrannini, 2009) (**figure 1**). As demonstrated by the Whitehall II study, insulin sensitivity is decreased up to 87% during the five years period before diabetes diagnosis. The fasting and the 2h postprandial glucose level increased linearly three years before T2D manifestation, and the β-cell function, determined by HOMA, showed first pathological increases three to four years before diabetes manifestation, followed by a decrease up to 63% until diagnosis (Tabak et al., 2009). Additionally, insulin resistance can also be observed in individuals with normal-glucose tolerance (NGT), in first degree relatives of subjects with type 2 diabetes, and in individuals with impaired fasting glucose (IGT) (Abdul-Ghani et al., 2006b; Weyer et al., 2001; Lillioja et al., 1988; Saad et al., 1988) and predicts the development of glucose intolerance and T2D (Weyer et al., 2001; Abdul-Ghani and Defronzo, 2009).

Insulin secretion and β-cell function as contribuiting factors in the development of type 2 diabetes

The β-cells of the islet of Langerhans in the pancreas secrete insulin dependent on actual blood glucose concentrations. In insulin-resistant states, β-cells produce increased amounts of insulin to counteract resistance to the hormone in peripheral tissues (**figure 1**). When β-cells can no longer compensate the insulin resistance by an hyperinsulinemic state, hyperglycemia occurs and T2D becomes clinically manifest (Festa et al., 2006; Abdul-Ghani and Defronzo, 2009; Abdul-Ghani et al., 2006a; Abdul-Ghani et al., 2006b; Kahn et al., 1989; Kahn et al., 1993).

In an animal model this concept has been confirmed, demonstrating that knockout of the peripheral muscle insulin receptor did not induce a diabetic phenotype (Lauro et al., 1998), indicating that up-regulation of β-cell activity compensates for insulin resistance and may engender normal fasting glucose levels by hyperinsulinemia. In contrast, it has been shown, that a β-cell defect in the presence of insulin resistance results in a diabetic phenotype (Bergman, 1989; Bruning et al., 1997).

Furthermore, the development of β-cell dysfunction is associated with the consumption of high fat diet, which may be accompanied with higher levels of endogenous FFA. FFA, in particular saturated FFA, are potentially lipotoxic to pancreatic β-cells, promote apoptosis

(Unger, 2002) and have an impact of cytokine secretion of blood cells (Simon et al., 2013).

The gastrointestinal hormones and the incretin effect

Oral glucose administration induces a much greater degree of insulin secretion compared to a parenteral isoglycemic intravenous glucose infusion, suggesting that gastrointestinal hormones, the incretins play an important role (MCINTYRE et al., 1964; MCINTYRE et al., 1965). Gastrointestinal hormones like glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are essentially involved in the regulation of gastric acid secretion and gut motility, release of pancreatic enzymes, and nutrient absorption. The incretin GLP-1, support the disposal of glucose through the stimulation of insulin secretion from the pancreas. This incretin effect is of relevance in the glucose metabolism and applied in the treatment of T2D (Drucker, 2006; Drucker and Nauck, 2006; Nauck et al., 2004; ELRICK et al., 1964) since it has been described that the overall incretin effect in T2D with deteriorated glycaemic control is reduced (Nauck et al., 1986; Nauck et al., 1993; Calanna et al., 2013).

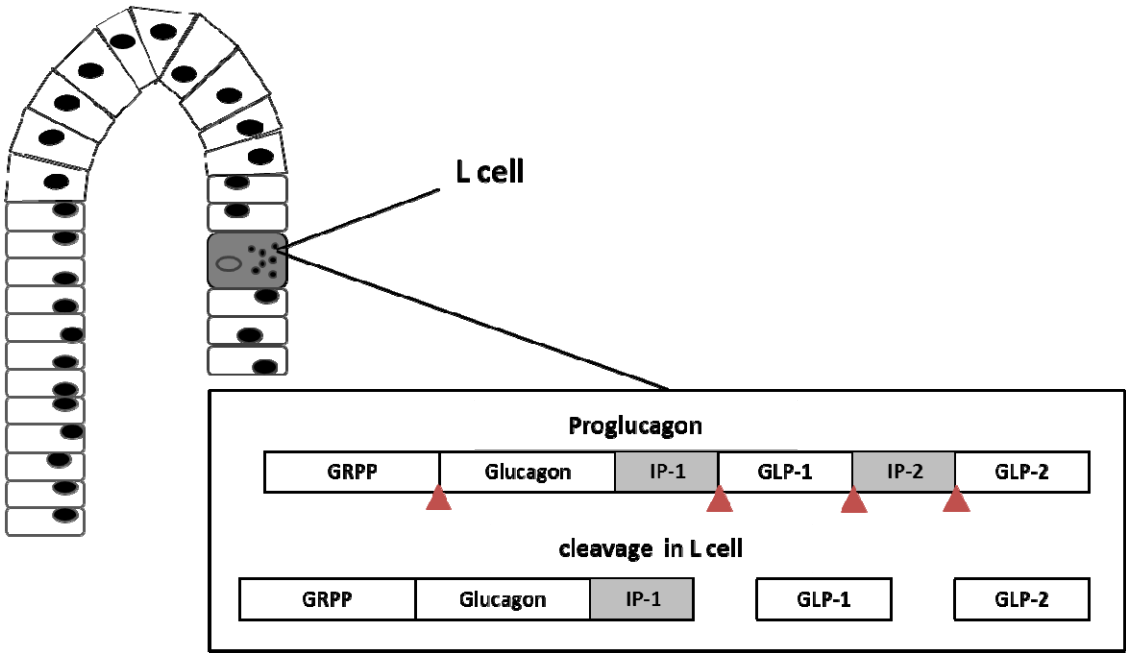

Fig. 2 Schematic presentation of proglucagon-processing
Modified from (Rowland and Brubaker, 2008): Synthesis of the 160 amino acid precursor protein by the intestinal L-cell. The hormone Glucagon is part of the sequence but is not liberated in his active form from the intestinal L-cell.

GIP is synthesized from the enteroendocrine K cells, has insulinotropic effects and only slight effects on the gastric acid secretion (Dupre et al., 1973). It was the first identified incretin, followed by GLP-1.

GLP-1 and GLP-2 are co-secreted after food intake from intestinal L-cells (**figure 2**) and are rapidly degraded by dipeptidyl-peptidase (DPP)-4. The incretin release is interdependent on gastric emptying and blood glucose (Samsom et al., 2009; Drucker, 2006). Defects of the incretin system in patients with T2D have been tackled by medical treatment with incretin analogues or mimetics as well as treatment with DPP-4 inhibitors, all of which improve glucose metabolism. GLP-1 improves insulin secretion, inhibits glucagon action, has effects on central-nervous-system, and inhibits gastric emptying (Drucker and Nauck, 2006; Drucker, 2002).

GLP-2, an intestinotrophic peptide, enhances intestinal epithelial barrier function by affecting both para-cellular and trans-cellular pathways. GLP-2 treatment has been shown to increase intestinal weight and mucosal thickness, surface area and cryptic architecture in animals and humans (Rowland and Brubaker, 2008; Jeppesen et al., 2005). Furthermore, chronic administration of GLP-2 also affects intestinal functions towards an increase in nutrient digestion and absorption as well as in barrier function in normal mice (Benjamin et al., 2000; Brubaker et al., 1997; Cheeseman, 1997; Kato et al., 1999; Drucker, 2002). It has been proposed to treat patients with chronic bowel disease with GLP-2 in a clinical setup, as it has been shown to reduce inflammation and enhance mucosal integrity in several injury models in rodents. However, as GLP-2 affects cell proliferation and differentiation through insulin-like growth factor (IGF)-1 related pathways. Therefore, it was proposed to closely monitor patients with known neoplasms during treatment with DPP-4 inhibitors, since it has been described that DPP-4 degraded both peptides, GLP-1 and GLP-2 (Rowland and Brubaker, 2008; Masur et al., 2006).

Intestinal microbiota and the impact on host metabolism

Numerous animal studies point to a link between intestinal microbiota, intestinal permeability, and chronic inflammation (Sommer and Backhed, 2013; Tremaroli and Backhed, 2012). Furthermore mainly in mouse models it has been supposed that intestinal microbiota are involved in the regulation of metabolic parameters (Musso et al., 2010; Lye et al., 2009; Cani and Delzenne, 2009b; Cani et al., 2009b) including insulin resistance (Genta et al., 2009), postprandial glucose response (Cani et al., 2009a),

obesity (Parnell and Reimer, 2009) and type 2 diabetes (Qin et al., 2012). However, the majority of these studies were performed in animal models, while data from human studies are rare.

Gut microbiota and gut hormones (incretins)

Recently, in rodents it has been demonstrated that the alteration of gut microbiota composition affects the intestinotrophic gut hormone glucagon-like peptide (GLP)-2 (Cani et al., 2009b) as well as the insulinotropic GLP-1, GIP and PYY (Cani et al., 2009a).

Cani et al. showed that mice treated with prebiotics leads to have increased GLP-2 levels resulting in reduced gut permeability, supposed to increased expression of the tight junction proteins like occludin and zona occludens-1 (Cani et al., 2009b; Moran et al., 2012). The increased GLP-2 release was likely mediated by changes of FFA levels in the gut, in particular the SCFA butyrate (Bartholome et al., 2004; Tappenden et al., 2003), which is one of the SCFA produced during the fermentation of the prebiotics by the gut microbiota (Roberfroid et al., 2010; Ramnani et al., 2012).

Gut microbiota and systemic inflammation

Another mechanism how defined modulation of the gut microbiota may prevent metabolic diseases could be by reducing the translocation and systemic concentrations of the endotoxin Lipopolysaccharide (LPS), leading to an altered inflammatory status. It is well established that elevated levels of LPS, a component of the outer cell wall of gram-negative bacteria, in the peripheral blood are associated with low-grade inflammation (Caradonna et al., 2000; Cani et al., 2012; Cani and Delzenne, 2009c; Andreasen et al., 2010b). LPS is a dominant exogenous Toll-like receptor (TLR-)4 ligand, which activates the innate immune system (Medzhitov, 2007; Medzhitov, 2001). In an animal study by Cani et al., decreased intestinal permeability modulated by prebiotic treatment correlated with lower portal plasma LPS concentrations known to trigger inflammation and oxidative stress (**figure 4**). In these mice improved systemic and hepatic inflammation was shown (Cani et al., 2009b).

Since both, cytokines and oxidative stress have been shown to be potentially harmful to insulin-producing β-cells, this could be an additional effect. Low-grade inflammation and differentially regulated cytokines have been reported in human subjects with diabetes to associate with insulin resistance, decreased β-cell function, impaired glucose homeostasis, prediabetes, diabetes and obesity (Herder et al., 2009b; Herder et al., 2009a; Pfleger et al., 2008a; Herder et al., 2007b; Herder et al., 2007a; Herder et al.,

2005a; Schloot et al., 2007; Kempf et al., 2007). In mice an additional role of leptin, an adipokine secreted from the white adipose tissue (Xie et al., 2008), mainly involved in energy metabolism (Mantzoros et al., 2011) has been described to alter the intestinal translocation of bacteria by modulating the bacterial adherence to the intestinal mucosa (Amar et al., 2011). Those observations, mainly from animal studys, indicate that the gut microbiota is in important factor to contribute to systemic inflammation and may contribute to changes in insulin sensitivity, possibly also in humans.

Metabolic effects of modulated microbiota

In animal studies altered gut microbiota has been associated by diseases like obesity and diabetes and is influenced by weight alterations and dietary intervention (Cani and Delzenne, 2009a; Cani et al., 2008b). Ingestion of probiotics in animal experiments and in only few human clinical trials has been shown to influence gut microbiota composition (Ley et al., 2005; Turnbaugh et al., 2006), intestinal permeability, blood glucose, HbA1c, triglyceride and LDL-cholesterol levels (Cani et al., 2007b; Yadav et al., 2007; Hlivak et al., 2005), and blood pressure (Sipola et al., 2002; Seppo et al., 2003) (**table 1**).

However, in human subjects, the link between alterations of the gut microbiota by ingestion of probiotics and factors associated with diabetes or obesity such as insulin resistance, β-cell activity, low grade inflammation and altered incretin hormones is less well understood.

At present, it is poorly investigated whether probiotic treatment leads to an alteration of ectopic fat distribution which is defined by the deposition of triglycerides within cells of non-adipose tissue that normally contain only small amounts of fat (Lettner and Roden, 2008) focusing particularly on the intracellular lipid contents in skeletal muscle and liver, which are directly related to insulin resistance (Szendroedi and Roden, 2009).

Recently, a preliminary study with ten healthy human subjects has shown that the intake of prebiotics over a period of two weeks lead to increased plasma GLP-1 and Peptide YY concentrations as well as decreased post prandial glucose concentrations in humans (Cani et al., 2009a). To date only one other human study addressed whether the alteration of gut microbiota by ingestion of probiotics can influence glucose homeostasis and insulin sensitivity, but whether a beneficial effect of probiotics on glucose metabolism in humans indeed is related to improved gut integrity (via GLP-2) and to an amelioration of chronic systemic inflammation is unknown.

Table 1 Metabolic effects of probiotic strains administered to different animal models

Probiotic strain / combinations	Result of intervention	Animal model	Reference
L. casei	inhibited occurrence of diabetes modified immune response	NOD mice	(Matsuzaki et al., 1997b)
L.casei	plasma glucose ↓ modified immune response	NIDDM-KK-Ay mice	(Matsuzaki et al., 1997c)
L.casei	incidence of diabetes ↓ insulin secreting β-cells ↑	AXN-induced BALB/c mice	(Matsuzaki et al., 1997a)
L. rhamnosus GG	HbA1c and oxidative stress ↓ glucose tolerance ↑ insulin secretion ↑	STZ-induced diabetic rats	(Tabuchi et al., 2003)
L. reuteri GMNL-263	HbA1c↓ blood glucose ↓	STZ-induced diabetic rats	(Lu et al., 2010)
L. plantarum DSM 15313	blood glucose ↓	HFD C57BL/6J mice	(Andersson et al., 2010)
B. longum BIF CGMCC no. 2107	endotoxin ↓ intestinal inflammation ↓ intestinal growth factor ↑	HFD rats	(Chen et al., 2011)
B. animalis subsp. lactis 420	inflammatory status ↑ metabolic status ↑ bacterial translocation ↓	HFD vs. chaw diet C57bl6 ob/ob knock-out mice	(Amar et al., 2011)
L. acidophilus NCDC14, L. casei NCDC19	diabetic dyslipidemia↓ lipid peroxidation ↓	STZ-induced diabetic rats	(Yadav et al., 2008)
L. acidophilus NCDC14, L. casei NCDC19	HbA1c and blood glucose ↓ free fatty acids ↓ triglycerides ↓	Fructose-induced diabetic rats	(Yadav et al., 2007)
L. acidophilus, L. rhamnosus B. lactis,	impoved blood glucose due to altered gliclazide transport across ileal tissue	AXN-induced diabetic rats	(Al-Salami et al., 2008)
VSL#3	β-cell destruction and insulitis ↓ IL-10↑	NOD mice	(Calcinaro et al., 2005)
VSL#3	insulin resistance ↓ hepatic steatosis ↓ inflammation ↓	HFD C57BBL-6 mice	(Ma et al., 2008)

Different Lactobacilli (L.) and Bifidobacteria (B) species and their metabolic effects. Streptozotocin (STZ), Alloxan (AXN), non-obese diabetic (NOD) mice, non-insulin-dependent diabetes mellitus (NIDDM) model, high fat diet (HFD), ↑ means improved / increased, ↓ means reduced / decreased. VSL#3 is patented probiotic mixture, containing *Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus bulgaricus and Streptococcus thermophilus.*

A recent study by Vrieze et al. investigated the effects of allogenic fecal transplantation of lean donors on insulin sensitivity to subjects (n=18) with newly diagnosed metabolic syndrome (Vrieze et al., 2012). The fecal infusion from lean donors improved insulin resistance as well as fasting lipid levels in obese individuals with metabolic syndrome. These results underscore the potential role of gut microbiota on glucose and lipid metabolism (Vrieze et al., 2012). Interestingly, donor-feces infusion has recently been shown to result in 81% (13 of 16 patients) in resolution of *Clostridium difficile* associated diarrhoe whereas antibiotic therapy with vancomycin results only in 31% (4 of 13 patients) in resolution (Van et al., 2013). This suggests that donor-feces infusion might be used as a potential therapeutic strategy against recurrent *C. difficile* infection, which is difficult to treat, even with antibiotics (Van et al., 2013). Besides transferring the complex community of microorganisms, with poor knowledge of the composition and diversity, the ingestion of probiotics might be an alternative (Gerritsen et al., 2011).

A study with a heterogenous group of participants of 54 males with T2D or with impaired or normal glucose tolerance who received *L. acidophilus* NCFM for 4 weeks showed preserved insulin sensitivity compared to the placebo group, but no effect on the systemic inflammatory response (Andreasen et al., 2010a). There was considerable variability among study participants and it was not reported whether those with improved insulin sensitivity were diabetic or normoglycemic.

Recently, Ejtahed et al. showed that the consumption of a probiotic yogurt containing *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 over six weeks improved enzymatic antioxidant status, fasting blood glucose level and the HbA1c in patients with type 2 diabetis compared to the control group which consumed conventional yogurt (Ejtahed et al., 2012). The absence of a control group that consumed no yogurt, even in regard to the described biological activities of milk proteins (Korhonen and Pihlanto, 2006) could limit the generalizability of these results (Ejtahed et al., 2012).

Lactobacillus reuteri

Probiotic treatment with Lactobacilli and Bifidiobacteria are supposed to benefit of the host metabolism in different ways, involving preventative and therapeutic aspects (**table 1**).

Probiotics such as *Lactobacillus (L.) reuteri* has been shown to improve diseases such as infant colic (Savino et al., 2007; Indrio et al., 2008), IgE-associated eczema (Abrahamsson et al., 2007; Bottcher et al., 2008) and diarrhea (Shornikova et al., 1997b; Shornikova et al., 1997a; Weizman et al., 2005). In animal models *L. reuteri* reduced

intestinal inflammation (Liu et al., 2010) and seemed to have an impact on immune reactivity measured *in vitro* (Livingston et al., 2010; Hoffmann et al., 2008).

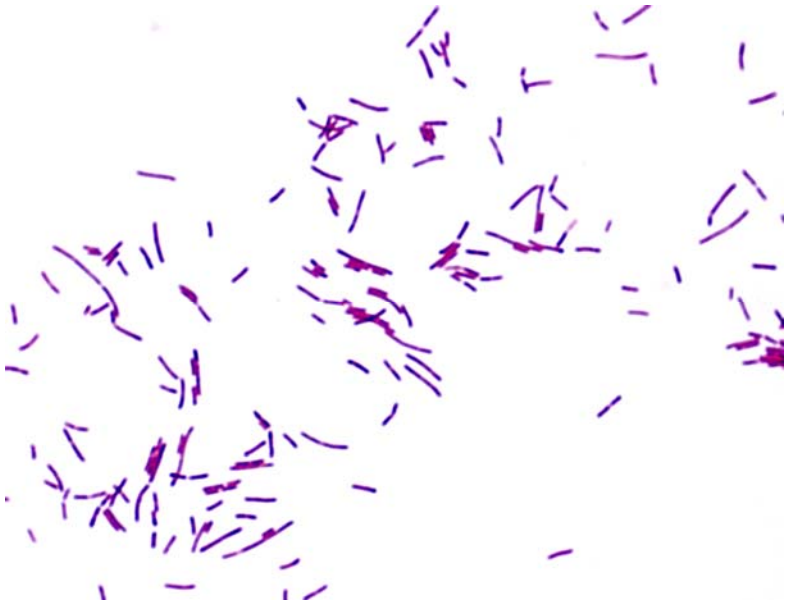

Fig. 3 ***Lactobacillus reuteri***
Colored in blue after a gram stain (100x)
(private source, foto made at the Institute for Medical Microbiology and Hospital Hygiene, Heinrich-Heine-University Düsseldorf, Germany 2012, M.C. Simon)

Lactobacillus reuteri is a gram-positive bacterium (**figure 3**), which also naturally inhabits the gut of mammals, and is used by the food industry to prepare probiotic nutrients. *L. reuteri* has been tested for host tolerance in children (Ruiz-Palacios et al., 1992), healthy adults (Wolf et al., 1995), and immunosuppressed patients with HIV (Wolf et al., 1998). One of the best documented effects of *L. reuteri* is the treatment of rotavirus-induced diarrhea. Treatment of rotaviral diarrhea by consumption of *L. reuteri* significantly shortens the duration of the illness compared to placebo treatment in young children between 6 and 36 months of age receiving up to 10^{11} colony-forming units (CFU). This effect was dose-dependent: the more *L. reuteri* is consumed, the faster the diarrhea stops (Shornikova et al., 1997a; Shornikova et al., 1997b).

Furthermore*, L. reuteri* is found in breast milk (Sinkiewicz and Nordström, 2005), and oral intake of the mother likewise increases the amount of *L. reuteri* present in her milk, and thus the likelihood that it will be transferred to the child (Abrahamsson et al., 2005). *L. reuteri* benefits its host in a variety of ways, particularly by modulating the immune system and has been shown to be safe in application, even in neonates at dosages up to 10^{10}

CFU per day. *L. reuteri* has a well-studied safety and a strong probiotic activity, when compared with 46 other strains of Lactobacillus spp. (Jacobsen et al., 1999).

Overall, gut microbiota consisting of complex communities of microorganisms that colonise the intestine seem to have an major impact on health and disease (Gerritsen et al., 2011) and as recently shown in a metagenome-wide association study, patients with T2D are characterized by a moderate but statistically highly significant degree of gut microbial dysbiosis (Qin et al., 2012).

So far, the above mentioned studies demonstrate that alteration of intestinal microbiota may affect the host's metabolic status. However most of the studies investigating the effects of prebiotic or probiotic alterd microbiota were performed in animal models. To understand the potential impact of probiotic bacterial strains on the composition of the human gut microbiota and on the host's metabolic and immunological status, further carefully controlled studies in humans are required.

Hypothesis

The goal of the study was to test the hypothesis that *L. reuteri*-enriched microbiota improve insulin sensitivity and insulin secretion in lean and obese glucose tolerant subjects by improving GLP-2 and / or GLP-1 linked insulinotropic effects.

Specific aims

The specific aims were therefore:

To investigate the effects of *L. reuteri*-enriched gut microbiota in a prospective, double-blind, placebo-controlled randomized trial over 8 weeks in human subjects on

I. insulin sensitivity and β-cell function
II. incretin concentrations
III. inflammatory status and reactive oxygen species
IV. ectopic fat distribution

These aims were addressed by the following work packages (WPs; ***figure 4****):*

1.WP: *Influence of L. reuteri enriched microbiota on insulin sensitivity, β-cell function and glucose tolerance:* Oral glucose tolerance test (OGTT) provided information on glucose tolerance, and was performed in all subjects before, during and after study. Furthermore, OGTT provided information of the β-cell function, based on insulin, C-peptide secretion and mathematical model calculations (e.g. disposition index, adaptation index). Whole body insulin sensitivity was assessed by the gold standard, the hyperinsulinemic-euglycemic clamp, in combination with a tracer to assess the hepatic insulin sensitivity.

2.WP: *Influence of microbiota on incretins:* It was tested whether administration of *L. reuteri* affects gut hormone secretion of intestinotrophic GLP-2, insulinotrop GLP-1 and GIP. An OGTT was performed and incretin secretion over time was determined during the OGTT up to 180 min after glucose ingestion. Blood glucose, insulin, and incretin concentrations from OGTT were compared to the results of an isoglycemic intravenous (i.v.) glucose infusion test to address the incretin effect *in vivo* (Nauck et al., 2004; Meier et al., 2001). Gastric emptying influences the release of gut hormones and may be disturbed in obese subjects and patients with manifest T2D. To take this into account we performed a ^{13}C-octanoic acid breath test in each participant. Additionally, the levels of lipopolysaccaride (LPS), which are associated with intestinal permeability, were measured in peripheral blood.

3.WP: *Inflammatory status and reactive oxygen species*: Inflammatory status and reactive oxygen species (ROS) were assessed by measuring pro-inflammatory (e.g. C- reactive protein (CRP), tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, IL-1β, macropahge inflammatory protein (MIP)-1β) and regulatory (e.g. IL-10, IL1ra, Interferon-gamma (IFN-γ)) cytokines and chemokine (monocyte chemotactic protein (MCP)-1, also known as CCL2). Serum concentration of thiobarbituric acid (TBARS) as a marker for reactive oxygen species (ROS) was determined. Systemic LPS-concentrations were determined by the limulus amebocyte lysate (LAL)-Test from serum samples. Furthermore, the concentrations of total free fatty acids (FFA) in the blood were measured.

4.WP: *Ectopic fat distribution:* The quantification of hepatocellular and myocellular triglycerides content from ^{1}H-MR spectroscopy (MRS) was measured relative to intracellular water content, and calculated as described (Krssak et al., 2004b; Krssak et al., 1999; Hwang et al., 2007). Spectra were acquired and processed to assess content of intramyocellular lipids using jMRUI or NUTS software according to methods developed

and previously applied by the research group (Szendroedi et al., 2011). All magnetic resonance (MR) measurements were performed on a 3.0 Tesla (T) whole body clinical scanner (Philips achieva X-series, Best, The Netherlands).

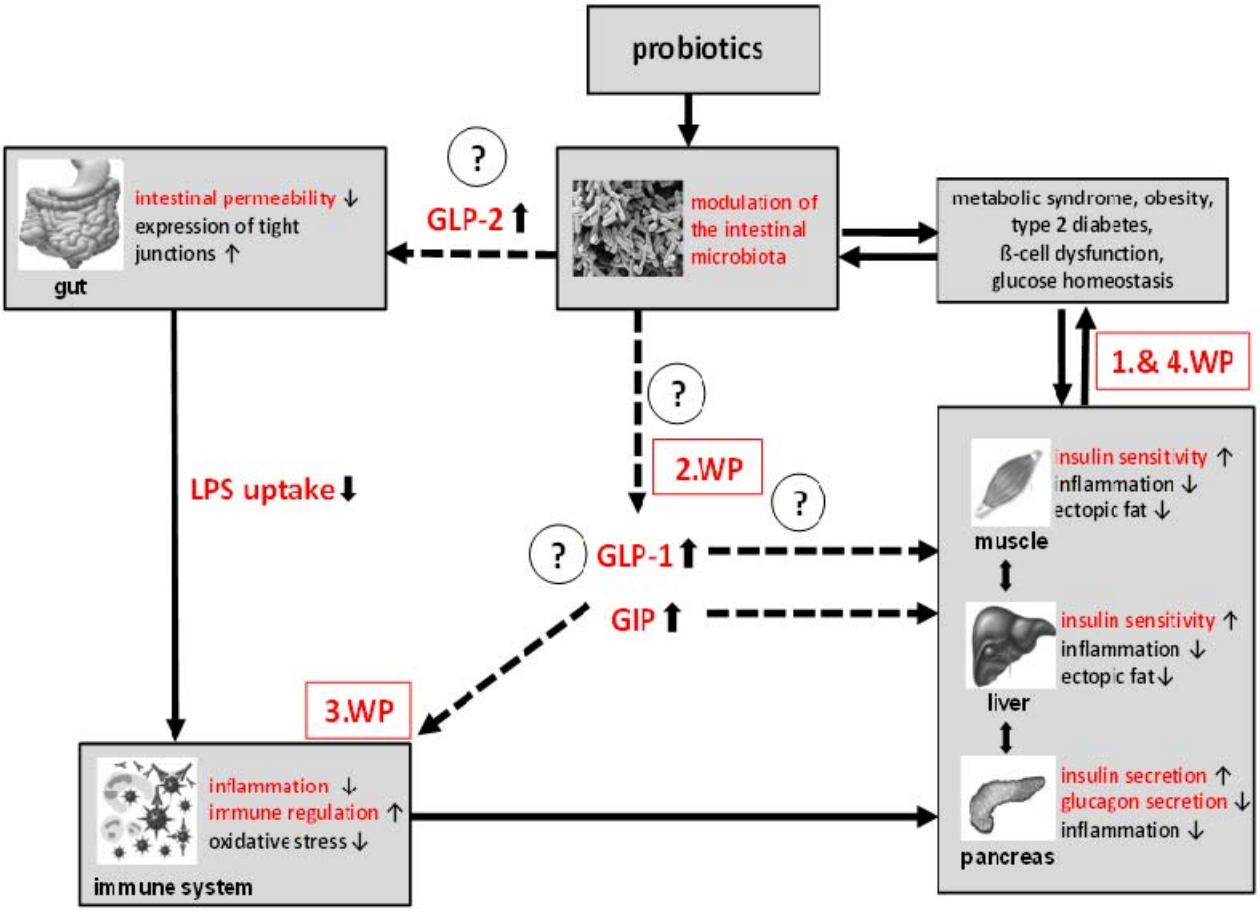

Fig. 4 Schematic overview of the probiotic effects
Specific questions or work packages (WPs) which have been addressed are marked red. Continuous lines indicate likely pathways, while the dotted lines represent putative pathways. ↑ means improved and ↓ means decreased effects. For further details see main text.

Material and Methods

Study design

We performed a double-blind, placebo-controlled, randomized, prospective, longitudinal descriptive trial in 10 glucose-tolerant obese participants and 11 healthy lean control subjects (matched for age and sex) recruited at the German Diabetes Center (DDZ) in Düsseldorf. The study was performed according to the Declaration of Helsinki. All subjects provided written informed consent. The single centre study was approved by the ethics committee of the University of Duesseldorf and registered at ClinicalTrials.gov Identifier NCT01250106.

After a run-in phase, subjects received *Lactobacillus reuteri* at a dosage of $2*10^{10}$ organisms, equivalent to microbiota contained in two yogurts a day. As fermented milk products contain microbiota, it was mandatory for participating subjects to abstain from eating or drinking fermented milk products, especially other probiotic products, during the 8-week study period. A schematic workflow of the trial is illustrated in **figure 5**.

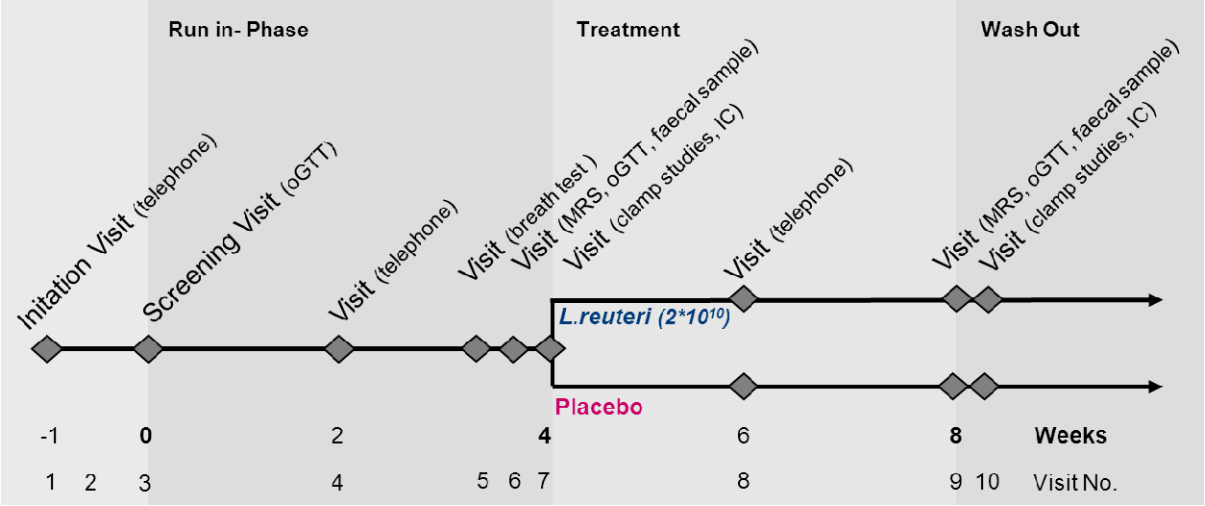

Fig. 5 Overview of the study design and frequency of visits.
Clamp studies included hyperinsulinemic-euglycemic clamp with [6,6-$^2H_2$]glucose tracer to assess peripheral and hepatic insulin sensitivity and isoglycemic i.v. glucose infusion to measure the incretin effect. Collection of faecal samples for further analysis, oral glucose tolerance test (OGTT), magnetic resonance spectroscopy (MRS), and indirect calorimetry (IC) were performed twice during study.

Study participants underwent a run-in phase of 4 weeks after the baseline screening visit during which all subjects received placebo. The run-in phase allowed evaluation of the effect of participation in the study e.g. on T2D risk factors. Food intake was monitored by a validated food frequency questionnaire every four weeks.

Type 2 diabetes related metabolic parameters, (gut hormones GLP-2 (non-insulinotropic), GLP-1 and GIP (insulinotropic), insulin resistance, β-cell activity (according to Mari 2001), body weight, body mass index (BMI), waist circumference, hsCRP, triglycerides (TG), cholesterol, LDL-/ HDL-cholesterol, cytokines and chemokines) were measured at the time when OGTT was performed. Isoglycemic intravenous glucose infusion and hyperinsulinemic-euglycemic clamp was performed within three days after OGTT.

After 4 weeks, subjects were randomised 1:1 into a placebo and a verum arm in a double-blind fashion and provided with capsules containing placebo or 10^{10} *Lactobacillus reuteri* (Nutraceutix, Redmond, WA, USA). They ingested one capsule of placebo or verum in the morning and one in the evening daily for 4 consecutive weeks.

Samples of feces for analysis of gut microbiota were obtained at the start of intervention (week 4, end of run-in phase) and at the end of the trial (week 8). Treatment adherence was assessed by counting capsules and by screening for *Lactobacillus reuteri* in gut microbiota using quantitative real-time PCR.

Study participants

Inclusion criteria

Inclusion criteria were an age range of 40 – 65 years, non-smoking, absence of gastrointestinal disease, willingness to abstain from intake of fermented milk products over a study period of 8 weeks, stable dietary habits at least within the last three months. Obese subjects were included with BMI 30-45 kg/m^2 and healthy lean control subjects with BMI 19-25 kg/m^2, matched for sex and age. All subjects had to be glucose tolerant and need to show stable fasting blood glucose levels as determined at screening and at the start of intervention.

Exclusion criteria

Subjects were excluded in case of pregnancy, cancer, chronic diseases, antibiotic therapy, competitive athletes and impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) at screening visit.

Subjects showing a strong loss in weight (defined as more than 5% loss of body weight during run-in) and changes in or onset of pharmacological treatment, especially the use of antibiotics due to infectious diseases, during run in phase were excluded from the study.

Anthropometric parameters

Body weight, body height, waist circumference and blood pressure were measured by standard operating procedures (SOP) Version 1.1, 2009 of the German Diabetes Center at each visit. In brief, body height and body weight were measured in standing position, feet next to each other, using calibrated Seca®-scales (Hoff Waagen, Essen, Germany) with light clothing, without shoes. Waist circumferences were measured with nonflexible tape. The tape horizontally surrounded the subjects body between proximal costal arch and distal iliac crest. The subjects were instructed to stand with their feet pointing forwards and approximately 25-30cm apart and to breathe out gently while the measurement was taken. Measurements were recorded in centimetres and as two independent measurements on each subject, and should not differ by more than 0.2 cm to ensure reproducibility. Blood pressure and pulse were measured using digital Omron M4-1® (Omron® Helathcare Europe B.V., Hoofdorp, The Netherlands) after removing restrictive clothing from the arm and on participant's heart level with the palm of the hand facing upwards, while the subject was sitting in a comfortable, calm position.

Bioelectrical impedance analysis (BIA, Nutriguard-S, Data Input GmbH Darmstadt, Germany, software Nurti 3 Version 3.0.88, P&P Trendmedia GmbH, Stockdorf, Germany) was used to measure body composition during study. BIA-measurements were performed in duplicates on each subject to ensure reproducibility and to calculate lean body mass and fat mass according to Segal (Segal et al., 1988).

Intestinal permeability in vivo

Alterations of the integrity of the intestinal barrier are involved in the pathogenesis of many diseases, e.g. inflammatory bowel diseases (IBD) and allergic intolerances to nutrients. In animal models a link between increased plasma endotoxin levels, released from the gut microbiota as a consequence of increased intestinal permeability, and obesity and insulin resistance has been described. Intestinal permeability might be the interface of the commensal microorganisms and endotoxins on one hand and the mucosal immune system on the other hand which need to balance between tolerance and immune response in the core of metabolic dysfunctions (Vajro et al., 2013; Scaldaferri et al., 2012; Teixeira et al., 2012).

Intestinal permeability is measured as the ability of two non-metabolized sugar molecules - mannitol and lactulose - to permeate the intestinal mucosa. Mannitol is easily absorbed and serves as a marker of transcellular uptake, while lactulose is only slowly absorbed and serves as a marker for mucosal integrity. To perform the test, lactulose and mannitol at defined amounts are mixed and ingested. The test measures the amount of lactulose

and mannitol recovered in a urine sample over the following 6 hours after intake. Initially, we attempted to measure gut barrier function by the before mentioned Lactulose-Mannitol-Test (Dastych et al., 2008; Bosi et al., 2006). However, we found a wide range of individual variation (>14%). Thus, our focus returned to serum parameters to measure gut barrier function, e.g. zonulin. The serum levels of the protein zonulin, which is a modulator of the intestinal permeability, are positively correlated with intestinal permeability and are detectable in peripheral blood (Sapone et al., 2006). Samples to determine zonulin concentrations were collected before (week 4) and after intervention (week 8). In cooperation with Prof. Alessio Fasano (Director of Mucosal Biology Research Center, University of Maryland School of Medicine, Baltimore, MD, USA), we aimed to use a newly validated human monoclonal antibody-based ELISA to measure serum zonulin concentrations. However, a thoroughly evaluated and validated assay has not yet been fully established with the end of this study (A. Fasano, personal communication).

Gastric emptying

Data of ^{13}C-octanoic acid breath test were used to assess disturbed gastric emptying and potentially exclude patients with pathologically delayed gastric emptying as there is a reciprocal interplay of gut hormones GLP-1 and GLP-2 and gastric motility (Meier et al., 2006; Guan et al., 2012; Wettergren et al., 1993; Nauck et al., 1997). Therefore, at the end of the run-in phase, each subject underwent a ^{13}C-octanoic acid breath test, which is well established at our institute (Ziegler et al., 1996). In brief, breath testing was performed after 12 h overnight fast and consisted of scrambled egg, white bread (60 g), margarine (5 g) and 150 ml water (total kcal 250). The tracer (100 mg ^{13}C-octanoic acid, euriso-top®, Saint-Aubin Cedex, France) was given in the egg yolk. Before, immediate after the test meal and in time intervals of 15 min, a breath sample of $^{13}CO_2$ recovery was taken and measured by infrared isotope analyser (IRIS, Wagner GmbH, Bremen, Germany).

The rationale of ^{13}C-octanoic acid breath test (^{13}C-OBT) is to measure gastric emptying of solids based on (1) the retention of ^{13}C-octanoic acid in the solid phase of a standard test meal during its passage through the gastric environment, followed by (2) a rapid disintegration from the solid phase in the duodenum with (3) subsequent absorption of ^{13}C-octanoic acid, and (4) hepatic oxidation to $^{13}CO_2$ (Braden et al., 2006).

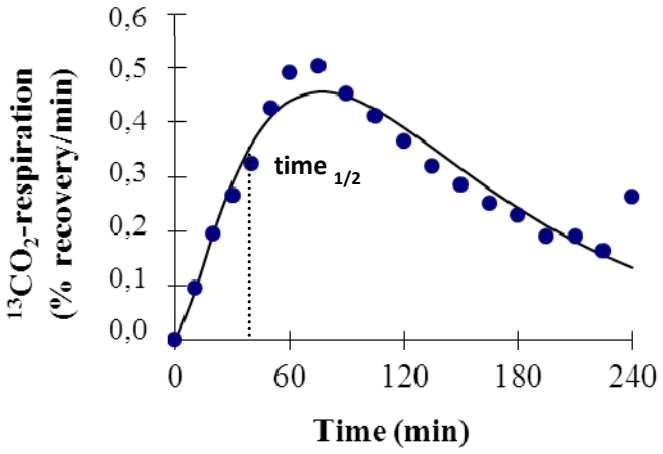

Fig. 6 Gastric emptying rate
Normal gastric emptying rate of one participant, exemplarily. Every dot represents one measurement of $^{13}CO_2$ recovery in the breath after oral administration of a ^{13}C-octanoic acid test meal. The half time of exhaled $^{13}CO_2$ is 48 min and represents a normal gastric emptying.

In vitro validation studies have shown that in a gastric environment ^{13}C-octanoic acid is firmly retained in the yolk of scrambled egg used as test meal. Once the meal reaches the duodenal lumen, ^{13}C-octanoic acid is rapidly absorbed through the intestinal mucosa and oxidized to $^{13}CO_2$ in the liver. The appearance of $^{13}CO_2$ in breath after oral administration of ^{13}C-octanoic depends mainly on the gastric emptying of the egg yolk into the duodenum (rate limiting step, **figure 6**). The other metabolic steps (absorption and oxidation) do not influence the rate of $^{13}CO_2$ exhalation as shown by studies in which, after duodenal instillation of ^{13}C-octanoic acid, $^{13}CO_2$ appears in breath almost immediately with very little inter-subject variability (Perri et al., 2005).

Analysis of the faecal content

Compliance for taking *Lactobacillus (L.) reuteri* containing capsules was assessed by screening for the bacteria in faecal samples. For the species-specific quantification, real-time PCR to detect total bacterial load (Euba) (Yang et al., 2002), enterobacteriaceae content (tuf), Lactobacillus spp. (Lac. spp.) (Byun et al., 2004), and *L. reuteri* (Haarman and Knol, 2006) was performed. The real-time PCR was established in cooperation with Prof. Birgit Henrich at the Institute of Medical Microbiology and Hospital Hygiene at the Heinrich-Heine-University.

In brief, faecal samples were processed within 24 hours after collection and stored at -20° C until further analysis. DNA extraction was performed using a BioRobot EZ1 machine (Qiagen, Hilden, Germany) according to the manufacturer's instructions (Bizhang et al.,

2011). All primers and probes used for species-specific real-time PCR were retrieved from the literature or configured via the Primer Express software (DNASTAR, Madison, WI, USA, **table 2**), and qPCR Mastermix No ROX, Cat.-No: RT-QP2X-03NR (Eurogentec, Seraing, Belgium) was used. PCR components were added to an end-volume of 25 µl per reaction mixture. Amplification of the DNA was carried out on CFX Cycler (Version 1.5.534.0511; BioRad, Munich, Germany), with the following cycling scheme: 50°C for 2 min and 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min.

To reduce the risk of contamination during the analyses, dedicated rooms, dedicated equipment, and sterile water (delta select, Dreieich, Germany) were used. To exclude a possible cross-reaction of the *L. reuteri* real-time PCR with DNA from other bacteria of fecal samples, we tested potential pathogens of fecal samples (*E. coli, S. saprophyticus, C. perfringens, H. pylori, P. aeruginosa, S. aureus, Campylobacter spp.*) in a concentration of 10^6 copies. None of the tested pathogens showed cross-reactivity or inhibition with the *L. reuteri* real-time PCR.

Table 2 Primer and Probes

Genus / Species	Primer / Probe	Sequence (5'-3')	Ampl.	Ampl.	Lit.
Total bacterial load (Euba)	P 891 F	TGG AGC ATG TGG TTT AAT TCG A	22 nt	159 bp	(Yang et al., 2002)
	P 1033 R	TGC GGG ACT TAA CCC AAC A	19 nt		
	P 1024 02 S	CAC GAG CTG ACG ACA RCC ATG CA	23 nt		
Escherichia coli (tuf)	tuf-F	TGG TCA GGT ACT GGC TAA GC	20 nt	78 bp	configured via the primer express
	tuf-R	TCT TTG GAC AGA ATG TAC ACT TCA	24 nt		
	tuf-S	CCA TCA AGC CGC ACA CCA AGT TCG	24 nt		
Lactobacillus spp (*Lac. spp*)	Lacto-F2	TGG AAA CAG ATG CTA ATA CCG	21 nt	235 bp	(Byun et al., 2004)
	Lacto-R2	CGT CCA TTG TGG TAG ATT CCC T	22 nt		
	Lacto-S	CTG AGA CAC GGC CCA WAC TCC TAC GG	26 nt		
L. reuteri	F_reut_IS	ACC GAG AAC AAC GCG TTA TTT	21 nt	95 bp	(Haarman and Knol, 2006)
	R_reut_IS	CAT AAC TTA ACC TAA ACA ATC AAA GAT TGT	30 nt		
	P_reut_IS	ATC GCT AAC TCA ATT AAT	18 nt		

Primers and Probes were synthesized by Metabion (Martinsried, Germany)

To quantify the bacterial DNA load of each sample, we generated PCR-standards with distinct DNA-copies. We used pCR 2.1TOPO-Vektor (3,931kb, Invitrogen, Carlsbad, CA, USA) to clone the species-specific amplicons of each real-time PCR. After transformation of competent *E. coli*, plasmid-containing *E. coli* clones were grown on LB-Amp plates.

Plasmid-DNA of positive clones was prepared by using the High Pure Plasmid Isolation Kit (Roche Diagnostics, Mannheim, Germany), and as a control of insert length digested with using the restriction enzymes Xho I und Hind III (Fermentas, St. Leon-Rot, Germany). A mixture of 10 µl restriction mix and 2 µl of sample-buffer were separated on 2% agarose gel to determine the length of the inserted fragments. Clones with the anticipated insert length (**table 2**) were used for preparation of standards containing 10^5 - 10 copies of plasmid, for quantification.

In preliminary experiments we found that the genus-specific Lac. spp PCR and the species-specific *L. reuteri* PCR were not comparable due to the different lengths of the resulting amplicons. Therefore, we decided to recalculate the pathogenic load of the samples. We recalculated the standards of *L. reuteri,* by using genomic DNA of *L. reuteri* in two dilutions, of which the genome equivalents were estimated in Lac. spp qPCR.

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (OGTT) was performed in the morning after an overnight fast with 75 g glucose ad 300 ml water (Dextrose O.G-T., Roche Diagnostics Deutschland GmbH, Mannheim, Germany). In blood samples drawn at different time points during the OGTT we measured the concentrations of blood glucose, insulin, C-peptide, glucagon, GLP-1, GLP-2, GIP, HbA1c, FFA, TG, immune mediators and reactive oxygen species (ROS, **figure 7, table 3**) to determine the metabolic status of subjects.

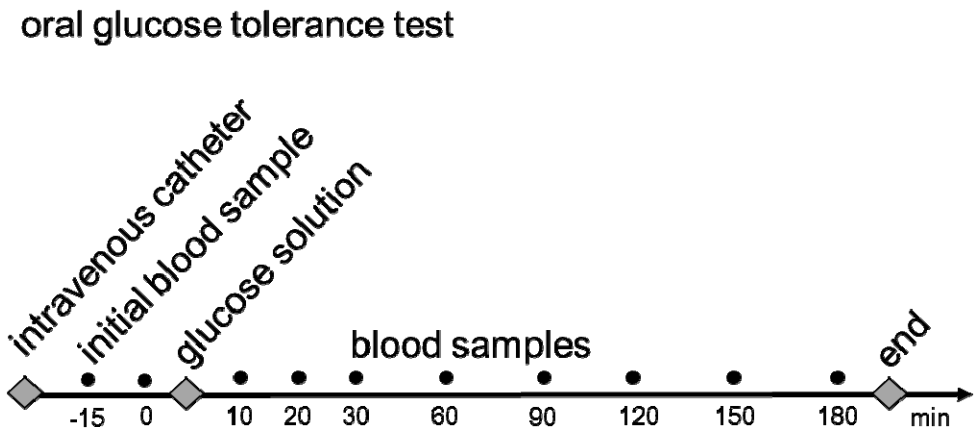

Fig. 7 Schematic representation of the oral glucose tolerance test (OGTT). Collection of two fasting blood samples was followed by immediate ingestion of glucose solution, containing 75 g glucose diluted in 300 ml water followed directly. Every black dot represents a time point for blood sampling. Investigated parameters from blood samples were concentrations of blood glucose, insulin, C-peptide, glucagon, GLP-1, GLP-2, GIP, HbA1c, free fatty acids, triglycerides, immune mediators, reactive oxygen species.

The parameters obtained from the OGTT also allow calculation of the oral glucose insulin sensitivity index (OGIS). The index is a validated method for the assessment of insulin sensitivity from the 3-hour oral glucose tolerance test with 75 g glucose. The OGIS provides information analogous to the index of insulin sensitivity (M-Value) obtained from the gold standard, the hyperinsulinemic-euglycemic clamp (Mari et al., 2001; Mari et al., 2005), which is described in a separate chapter (page 32).

Assessment of insulin secretion and β-cell function

Insulin secretion as a measure of β-cell function can be investigated by several metabolic tests. From measurements of plasma glucose, insulin, and C-peptide concentrations during the OGTT, β-cell function can be calculated. In cooperation with Prof. Giovanni Pacini, Padova, Italy we aimed to test β-cell activity by determining the disposition index (DI) (Kahn et al., 1993), area under the curve (AUC) insulin, and AUC glucose during the OGTT (Mari et al., 2001; Faerch et al., 2008; Thomaseth et al., 1996).

Moreover, β-cell function is assessable by the insulinogenic index (Mari et al., 2001; Mari et al., 2005). The interplay between insulin sensitivity and secretion can be described as the β-cells adaptive response to changes in insulin resistance (Ahren and Pacini, 2004). This interplay can be determined by the products OGIS × AUC_{CP} and OGIS × AUC_{INS}, where the AUCs are defined as the area under the concentration curves of C-peptide and insulin, respectively. These indices, called adaptation (AI) and disposition (DI) index were originally developed for the intravenous (i.v.) glucose test but have been proven to be useful determinants for the calculation of β-cell function from OGTT (Ahren and Pacini, 1997; Kahn et al., 1993; Pacini, 2006). Additonally, the DI was described as one of the most accurate physiologic parameters for the detection of changes of β-cell activity in the whole organism (Bergman et al., 2002).

Isoglycemic glucose infusion test

The rationale for performing the isoglycemic glucose infusion test is to measure the incretin effect (Nauck et al., 2004). The incretin effect is defined as the phenomenon that oral glucose administration induces a much higher degree of insulin secretion compared to a parenteral isoglycemic i.v. glucose infusion. This effect is caused by the release of incretins in response to the oral uptake of glucose (**figure 8**) (Drucker, 2006; Drucker and Nauck, 2006; ELRICK et al., 1964).

The isoglycemic glucose infusion test was newly established at the Clinical Research Center of the Institute for Clinical Diabetology at the German Diabetes Center.

Two catheters (Vasofix, Braun, Melsungen, Germany) were inserted into antecubital veins in the left and right arm for blood sampling and infusions, respectively. The test was performed in the morning after a 10 hours overnight fast. After drawing basal blood samples at -120, -15, -5 and 0 min, the test was started by an isoglycemic intravenous glucose infusion (20% in sterile water enriched with 2% [6,6-$^{2}H_{2}$]glucose) over 3 hours, using an Infusomat® Space (Braun, Melsungen, Germany). The glucose infusion rate was adjusted to the interpolated blood glucose concentrations achieved during OGTT.

Samples for blood glucose measurements were drawn every 2 min during isoglycemic intravenous glucose infusion test. The hyperinsulinemic-euglycemic clamp followed directly thereafter (**figure 9**).

To assess the incretin effect we calculated the AUC of insulin, C-peptide, glucagon, GLP-1, GLP-2 and GIP at each visit and compared the difference of ΔAUC before (week 4) versus ΔAUC after (week 8) intervention in the subjects of the verum and the placebo group, for each parameter (insulin, C-peptide, glucagon, GLP-1, GLP-2 and GIP, respectively). ΔAUC was defined as AUC of OGTT minus AUC of isoglycemic intravenous glucose infusion test. Missing values of insulin, C-peptide, GLP-1, GLP-2 and GIP at -15 min were replaced by values determined at 0 min and vice versa. Missing values during the time course were interpolated, and at 180 min extrapolated, by calculation of the mean in regard to the time-interval of the missing value. For GLP-1 (14 out of 756 values), GLP-2 (14 out of 756 values), glucagon (2 out of 420 values) and for GIP (14 out of 756 values) were calculated by interpolation or extrapolation.

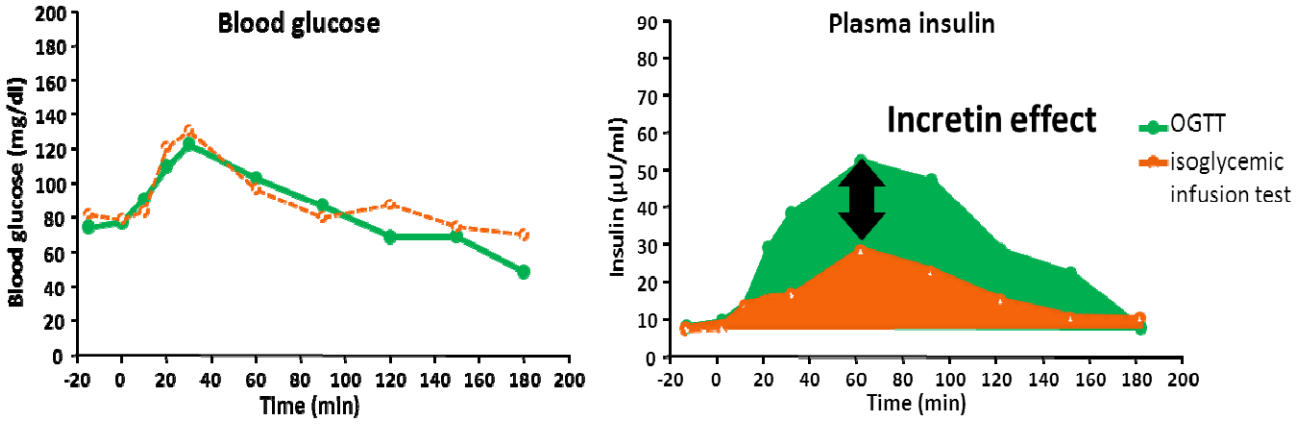

Fig. 8 The biological incretin effect determined by the isoglycemic clamp technique
Exemplarily, the blood glucose curve during OGTT (green) and isoglycemic i.v. glucose infusion (orange, left diagram) and the corresponding serum insulin levels (right diagram) of one participant. The area under the curve (AUC) of insulin determined during OGTT (green) minus the AUC of insulin determined during isoglycemic intravenous glucose infusion test (orange) indicate the incretin effect (black arrow).

Hyperinsulinemic-euglycemic clamp with isotopic dilution to assess insulin sensitivity

The gold standard to determine insulin sensitivity is the hyperinsulinemic-euglycemic clamp test. Combined with tracer substances this test allows to differentiate between hepatic and peripheral (muscle) insulin resistance. Moreover, the hyperinsulinemic-euglycemic clamp test allows to quantify the severity of insulin resistance (Defronzo et al., 1979). However, this method is difficult to implement in daily clinical practice. Therefore, several indices based on data from the oral glucose tolerance test and/or fasting blood sampling have been developed to assess insulin sensitivity (Armato et al., 2012; Matsuda and Defronzo, 1999; Anderwald et al., 2007). For example, the oral glucose insulin sensitivity index (OGIS) provides information of insulin-mediated glucose clearance, calculated from plasma glucose, insulin, and C-peptide concentrations during the oral glucose tolerance test (OGTT). The OGIS has been validated against the hyperinsulinemic-euglycemic clamp test and the results of the two methods show a high correlation (Mari et al., 2001; Mari et al., 2005; Ahren and Pacini, 2004). To reduce these cumbersome experimental clinical settings, further indices to determine insulin resistance have been established based on mathematical methods e.g. HOMA (homeostatic model assessment) (Wallace et al., 2004; Wallace and Matthews, 2002; Matthews et al., 1985; Mojiminiyi and Abdella, 2010; Levy et al., 1998; Geloneze et al., 2009) and QUICKI (quantitative insulin sensitivity check index) (Katz et al., 2000; Hrebicek et al., 2002; Chen et al., 2003).

In this clinical trial the gold standard hyperinsulinemic-euglycemic clamp test combined with isotopic dilution (6,6[$^2H_2$]glucose) was used to assess whole body and hepatic insulin sensitivity. In brief, after baseline blood sampling at -120 min, a primed (0.36 mg*(fasting blood glucose (mg/dl)/90 mg/dl)*body weight (kg)$^{-1}$* min^{-1} for 5 min), followed by a constant intravenous infusion (0.036 mg * body weight (kg)$^{-1}$ * min^{-1}) of the [6,6-$^2H_2$]glucose over two hours for assessment of endogenous glucose production (EGP) was given (**figure 9**). Directly after the isoglycemic glucose infusion test (180 min), a priming dose of short-acting human insulin (Insuman® Rapid, Sanofi-Aventis, Frankfurt, Germany) for 10 min was infused followed by a constant insulin infusion (40 mU/m^2 body surface area) over two hours, using Perfusor® Space (Braun, Melsungen, Germany). During this time blood glucose was adjusted to 80 mg/dl by a variable infusion rate of a 20% glucose solution, enriched with 2% [6,6-$^2H_2$]glucose to maintain the blood glucose concentration during the clamp test according to the HOTGINF protocol (Weickert et al., 2007; Nowotny et al., 2013). Throughout the hyperinsulinemic-euglycemic clamp blood samples were collected in 5 min intervals for the measurement of whole blood glucose concentration in

duplicates. The glucose infusion rates during the last 30 minutes of the clamp were used to calculate whole body insulin sensitivity (**figure 9**). Rates of EGP were determined from the tracer infusion rate of [6,6-$^2H_2$]glucose and its enrichment to the hydrogens bound to carbon 6 divided by the mean percent enrichment of plasma [6,6-$^2H_2$]glucose. Steady-state equations were appropriate for calculation of basal EGP and insulin-suppressed EGP during the last 30 min of clamp.

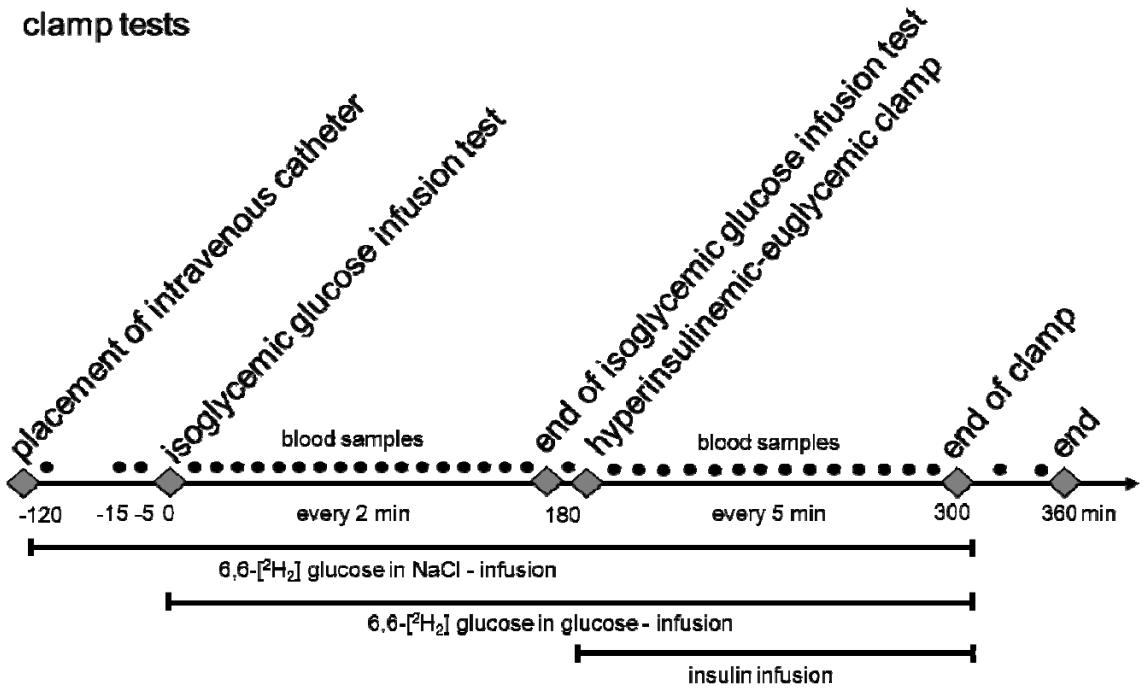

Fig. 9 Schematic representation of the time course of the clamp studies
Every black dot represents a time point for taking a blood sample. Investigated parameters from blood were blood glucose, insulin, C-Peptide, GLP-2, GLP-1, GIP, HbA1c, free fatty acids (FFA), triglycerides (TG), immune mediators, reactive oxygen species (ROS), atom percent enrichment of [6,6$^2H_2$] glucose (APEs)

Gas chromatography-mass spectrometry to determine atom percent enrichment of [6,6$^2H_2$] glucose

The atom percent enrichment (APE) of ^2H was determined by established methods according to standard operating procedures (SOP) of the gas chromatography-mass spectrometry (GC-MS) and hormone analytical laboratory at the German Diabetes Center headed by Ing. Peter Nowotny. After deproteinization, determination of APE of ^2H was done as described before (Krebs et al., 2001; Nowotny et al., 2013). In brief, 100 µl KF-EDTA plasma was diluted with an equal amount of water and deproteinized after adding 300 µl of 0,3 N $ZnSO_4$ solution followed by the addition of 300 µl of 0,3 N $Ba(OH)_2$ solution. After vortexing for 20 minutes, samples were centrifuged at 21.000 g at room

temperature. Thereafter, 400 µl of the sample were evaporated under a stream of nitrogen 5.0 at 37°C and both endogenous glucose and infused [6,6-$^{2}H_2$]glucose were derivatized with HOX (100 µl of 2% solution in pyridine, 60 min at 90°C, cooling for 5 min) and acetic anhydrate (200 µl, 60 min at 90°C, cooling for 5 min) to the aldonitrile-pentaacetate. The analyses were performed on a Hewlett-Packard 6890 gas chromatograph equipped with a 25 m/0.25 mm/0.12 µm CPSil5CB capillary column (Chrompack/Varian, Middelburg, Netherlands) and interfaced to a Hewlett Packard 5975 mass selective detector. Intra- and inter-assay variations were 0.6% and 1.0%.

Indirect calorimetry

The indirect calorimetry is a non-invasive method to determine *in vivo* the whole body energy expenditure and substrate oxidation by measuring the oxygen consumption (VO_2) and the carbon dioxide production (VCO_2) to calculate the respiratory quotient (RQ = VCO_2/VO_2) and resting energy expenditure (REE = $(3.941*VO_2+1.11*VCO_2)*1.44$) according to the abbreviated Weir equation.

Substrate oxidation was calculated according to Frayn (Frayn, 1983) with a fixed estimated protein oxidation rate (Pox) of 15% of REE: carbohydrate oxidation rate (mg/kg body weight*min) = $[(4.55*VCO_2) – (3.21*VO_2) – 0.459*Pox]*1000/kg$ bodyweight; where VCO_2 is carbon dioxide production and VO_2 is oxygen consumption; and lipid oxidation rate (mg/kg body weight*min) = $[(1.67*VO_2) – (1.67*VCO_2) – 0.307*Pox]*1000/kg$ bodyweight. Non-oxidative glucose disposal was calculated as Rd (rate of glucose disappearance, mg/kg body weight*min) minus carbohydrate oxidation (Nowotny et al., 2013). Due to an inherent variability of the instrumentation, case-related *in vitro* validation by individual calibration control evaluation (ICcE) was necessary. The *in vivo* rates were calculated, thereafter gaseous CO_2 and N_2 were infused into the hose of the metabolic cart and the mass-flow meters adjusted until the mean rates, observed in an individual subject, were exactly met. The resulting adjustment of the mass-flow regulator (in L/min, standard conditions, T=273°K, p=1013 hPa) was then taken to calculate the estimate for the true breath gas exchange rate of the subject (Schadewaldt et al., 2013).

Indirect calorimetry using canopy mode was performed using Vmax Encore 29n (CareFusion, Höchberg, Germany) at baseline and during steady state conditions of hyperinsulinemic-euglycemic clamp over a period of 20 min after 10 min adaptation time to the setting.

Biochemical analysis

The laboratory parameters, like blood cell count, HbA1c, triglycerides (TG), cholesterol, HDL-cholesterol, LDL-cholesterol and CRP, were determined by established methods according to SOPs of the gas chromatography-mass spectrometry (GC-MS) und hormone analytical laboratory at the German Diabetes Center.

Table 3 Blood sampling protocol of OGTT

blood collection scheme																
	tube and stabilizer	chilled	color of tube	[n]	[ml]	-15	0	10	20	30	60	90	120	150	180	ml
blood count	K-EDTA	/	/	1	1	X	instantly measure									1
HbA1c	K-EDTA	/	/	1	2	X	instantly measure									2
blood glucose	VB Lsg. (EKF)	/	/	10	1.5 (0.5+1)	X	X	X	X	X	X	X	X	X	X	15
insulin, C-peptide, cortisol	Serum	RT	blue	10	3.5	X	X	X	X	X	X	X	X	X	X	35
total FFA , TG , cholesterol	Na-EDTA + Orlistat	ICE	green	10	2 (2x1ml)	X	X	X	X	X	X	X	X	X	X	20
glucagon	Na-Hep + Aprotinin	ICE	red	5	2.5		X		X		X		X		X	13
GLP-1, GIP	DPP IV	ICE	purple	9	3	X	X	X	X	X	X	X	X		X	27
GLP-2	DPP IV	ICE	purple	5	6 (2x3ml)	X	X				X		X		X	30
immune mediators and CRP	Serum	RT	white	2	1.75/3.5		X								X	5,3
adiponectin, leptin	Serum	RT	yellow	1	1.75		X									1,8
retain sample	EDTA	RT	/	1	3		X									3
total (ml)																153
tubes (n)						5	6	2	2	2	4	2	4	1	5	33
tubes (ml)						15,5	19	6,5	6,5	6,5	12,5	6,5	13	3,5	16	105
syringe (ml)						3,5	6	3,5	6	3,5	6	3,5	6	3,5	6	48

Room temperature (RT), free fatty acids (FFA), triglycerides (TG), glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-(GLP)-1 and GLP-2, C-reactive protein (CRP), dipeptidyl peptidase-4 (DPP-4), stabilizer for FFA 40µl Orlistat and 20µl Na-EDTA, for glucagon 40µl Aprotinin and 20µl Na-Heparin

Briefly, fresh drawn blood samples were immediately chilled, centrifuged after 10 min and supernatants stored at -20°C until further analysis. Insulin, C-peptide, total free fatty acids (FFA) and glucagon were determined from venous drawn blood at baseline, during several time points of the 3 hours OGTT (**table 3**) and the hyperinsulinemic-euglycemic clamp test. The blood sampling protocols for OGTT and hyperinsulinemic-euglycemic clamp test was used initially for 3 pilot participants of each group. After verification of time points for blood sampling the scheme was adjusted (**table 3**).

Serum insulin was measured by a microparticle enzyme immunoassay (MEIA) on an AXSYM analyzer (Abbott, Abbott Park, IL, USA), C-peptide was measured by chemiluminescence immunoassay on an Immulite1000 system (Siemens, Erlangen, Germany) and plasma glucagon was analyzed by radio immune assay (RIA, Millipore, St. Charles, MO, USA). Plasma FFA concentrations were determined with the microfluorimetric method (Wako, Neuss, Germany) and cortisol was measured using a fluorescence polarization immunoassay on an Axsym analyzer (Abbott).
Venous whole blood glucose was measured in duplicate by Biosens C_line, GP (EKF diagnostic GmbH, Barleben, Germany) based on an electrochemical method using a chip-sensor technology (Nowotny et al., 2012). In brief, the β-D-Glucose in the samples was enzymatically cleaved in gluconic acid and hydrogen peroxide. The electrode measured the concentration of the hydrogen peroxide.

Incretin concentrations
Samples of peripheral blood for the determination of GLP-1, GLP-2, and GIP concentrations were collected into DPP-4-inhibitor containing tubes (BD P700, BD Biosciences, Franklin Lakes, NJ, USA) during OGTT and isoglycemic glucose infusion test. Measurements of all three gut hormones were performed in cooperation with the gas chromatography-mass spectrometry (GC-MS) und hormone analytic laboratory at the German Diabetes Center.
Concentrations of the gut hormone GIP were determined by using a gut hormone panel according to the manufacturer's instructions (human GIP (total) ELISA, Millipore, St. Charles, USA, CV 12%).
At first, concentrations of gut hormones GLP-1 and GLP-2 were determined by using a gut hormone panel according the manufacturer instructions (GLP-1 ELISA, TECOmedical, Sissach, Switzerland, CV 10%; GLP-2 ELISA, Uscn Life Science Inc., Wuhan, China, CV 15%, respectively). The manufacture instructions for GLP-2 measurements were changed by the company during the study period, requiring the use of other blood collection tubes and titration procedures. The required sample volume of EDTA plasma was not available, because we collected the blood into DPP-4-inhibitor containing tubes (BD P700, BD Biosciences, Franklin Lakes, USA). Additionally, the assay showed broad variance.

In a second step, we got the opportunity to get GLP-1 and GLP-2 measured by a well accepted and widely used method, as a part of a scientific collaboration with Prof J.J. Holst from the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen. The gut hormones GLP-1 and GLP-2 were measured as previously

described (Orskov et al., 1994; Hartmann et al., 2000; Deacon and Holst, 2009). In brief, GLP-1 and GLP-2 concentrations in plasma were measured by radioimmunoassays (RIA) after extraction of plasma with 70 % and 75% ethanol (vol/vol, final concentration), respectively. Carboxy-terminal GLP-1 immunoreactivity was determined using antiserum code no. 89390 which has an absolute requirement for the intact amidated carboxy-terminus of GLP-1 (7-36amide) and crossreacts less than 0.01% with carboxy-terminally truncated fragments and 89% with GLP-1 (9-36amide). GLP-2 was measured with an N-terminal specific antiserum, detecting only fully processed GLP-2 of intestinal origin. As standard, we used synthetic human GLP-2 and the tracer was rat GLP-2 with an Asp^{33} -> Tyr^{33} substitution. Sensitivity for both assays was below 5 pmol/l, and intraassay coefficient of variation below 10 %.

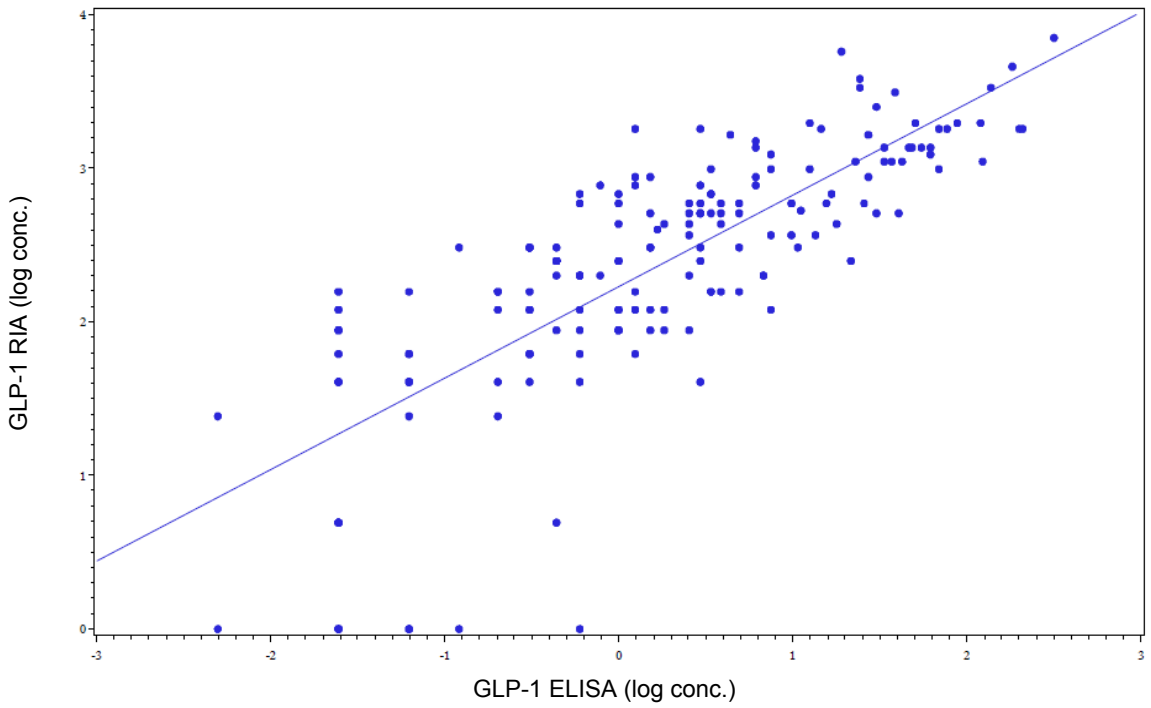

Fig. 10 Correlation of the GLP-1 concentrations detected by two different methods
Shown are the log GLP-1 values measured by ELISA (x-axis) and measured by RIA (y-axis), exemplarily at Visit 6. The log transformed GLP-1 values exhibit a linear relationship (r=0.778, p<0.0001). Regression Equation: log GLP-1 RIA = 2.23 + 0.60 * log GLP-1 ELISA.

Statistical analysis of the GLP-1 values, detected by the two different methods, showed a high correlation (r=0.778, p<0.0001, **figure 10**). In comparison, the log-transformed GLP-1 values, measured by the new radioimmunoassay (RIA) showed a smaller standard

deviation than the ELISA values (logGLP-1 ELISA CV 1.39, logGLP-1 RIA CV 0.94, respectively).

Reactive oxygen species (ROS)

Thiobarbituric acid-reactive substances (TBARS) measured from serum samples, reflect concentrations of oxidized lipids as markers of oxidative stress (Ristow et al., 2009). Measurements of TBARS were performed in cooperation with Dr. Gilles Séquaris of the research group energy metabolism of the Institute for Clinical Diabetology at the German Diabetes Center. Briefly, 50 µl serum were diluted in 50 µl 1.15% KCl solution and mixed with 0.8% thiobarbituric acid and 8.1% sulfosalicylic acid before TBARS extraction by butanol/pyridine (15:1). After separation of the butanol/pyridine layer by centrifugation (3000 g, 20 min, room temperature), the TBARS content (pmol/mg) was assayed on a Flx800 fluorometer (BioTek, Bad Friedrichshall, Germany).

Immune mediators and adipokines

Concentrations of pro-inflammatory (interleukin (IL)-6, IL-8, IL-1β, interferon-gamma (IFN-γ), tumor necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1β) and regulatory (IL-10, IL1ra) systemic cytokines and chemokines were determined by using a Flurokine MAP multiplex kit, human cytokine panel A (R&D Systems, Minneapolis, MN, USA) and quantified with Luminex technology (Bio-Rad Bioplex, Nazareth, Belgium) (Pfleger et al., 2008b; Pham et al., 2011). The adipokines leptin and adiponectin were determined by using the Flurokine MAP kit human obesity panel (R&D Systems, Minneapolis, USA) and measured by Luminex technology (Bio-Rad Bioplex, Nazareth, Belgium) as well (Pham et al., 2013). Following the manufacturer's instructions, color-coded microparticles with pre-coated analyte-specific antibodies were used to bind the analytes of interest. Detection of analytes was done by a microparticle-specific laser of the Bio-Plex-System, another laser determined the amount of analyte bound, because it measured the magnitude of the signal, which is in direct proportion. Standard curve and three control samples of two different dilutions were assayed in duplicates on all plates. All samples of one subject were measured together on one plate and in a combination of lean and obese subjects receiving verum or placebo. The mean intra- and inter-assay variance (CV) for all nine cytokines as well as for adiponectin and leptin was <15%. Detection limits were IL-6 0.50 pg/ml, IFN-γ 0.39 pg/ml, IL-8 0.42 pg/ml, TNF-α 0.61 pg/ml, IL-1β 0.34 pg/ml, MCP-1 0.11 pg/ml, MIP-1β 4.32 pg/ml and IL-10 0.38 pg/ml, IL1ra 1.6 pg/ml, adiponectin 124.28 pg/ml and leptin 81.58 pg/ml.

Limulus test to measure LPS

Serum endotoxin levels are a validated parameter to access gut permeability and inflammatory status (Cani et al., 2008a). Measurements of endotoxin were performed in cooperation with the research group immune modulation of the Institute for Clinical Diabetology at the German Diabetes Center headed by Priv.-Doz. Dr. Volker Burkard. Serum endotoxin (lipopolysaccharide, LPS) content was determined by the use of a chromogenic limulus amebocyte lysate (LAL) assay (QCL-1000 Lonza Ltd., Verviers, Belgium) according to the manufacturer's instructions with slight modifications (Habich et al., 2005). In brief, samples were centrifuged and diluted with pyrogen free water and heated for 15 minutes at 75°C. After pre-incubation of the plate for 10 minutes at 37 ± 1°C, all reagents, water blank, endotoxin standards, samples, and positive controls were carefully dispensed into the appropriate wells of the microplate and gently mixed before incubation. The reaction was stopped by the addition of 30% acetic acid and the extinction was measured by 405 nm photometrically on an ELISA-Reader Synergy 2 Gen5™ CL (BioTek, Bad Friedrichshall, Germany).

^{1}H MRS for the determination of hepatic and muscular fat content

All magnetic resonance imaging (MRI) and spectroscopy (MRS) were determined by established methods according to standard operating procedures (SOP) of the junior research group Metabolic Imaging headed by Dr. Jong-Hee Hwang at the Institute for Clinical Diabetology at the German Diabetes Center. All MRS measurements were performed on a 3.0 Tesla whole body clinical scanner (Philips Healthcare, Achieva, X-series, Best, The Netherlands) at DDZ. The quantification of liver and myocellular triglyceride content from ^{1}H-MR spectroscopy (MRS) was assessed relative to the internal water, and calculated as described previously (Krssak et al., 2004b; Krssak et al., 1999; Hwang et al., 2007; Krssak et al., 2010; Hamilton et al., 2011). The percentage of fat content was quantified as fat signals relative to the internal water peak at 4.7 ppm. The lipid peaks at -(CH_2)n - (1.3 ppm) and methyl -(CH_3) (0.9 ppm) were integrated by NUTS software (Acorn NMR, Livermore, CA, US).

Measurement of hepatocellular lipid content (HCL) by 1H MR spectroscopy

Using a fast spin echo sequence, MR images around the liver were obtained by using a navigation echo to remove breathing motion artifacts. To localize an appropriate voxel in the liver, transverse and coronal T2-weighted (T2w) images of 7 mm thickness were acquired. A Position Resolved Spectroscopy (PRESS) sequence with a repetition time of

4 s and an echo time of 35 ms was employed. Spectroscopic data was acquired in a volume of interest (VOI) of 25 x 25 x 25 mm^3 in the liver. The VOI were carefully placed to avoid major vessels and fat depots in the liver. Data was transferred to a desk computer and processed using NUTS software (Acorn NMR, Livermore, CA, USA).

Determination of intramyocellular lipid content (IMCL) by 1H MR spectroscopy

MR images of the left calf were acquired using a fast spin echo sequence. To choose a voxel in the soleus and tibialis anterior muscles, transverse, coronal and sagittal T1-weighted (T1w) images of 4 mm slice thickness were acquired to clearly visualize fat depots. Again, a VOI was carefully placed to avoid muscular fat depots. A PRESS sequence with a repetition time of 2 s and an echo time of 31 ms was used. Spectral data was acquired with and without water suppression (CHESS) for a VOI of 12 x 12 x 12 mm^3. Spectra were processed to assess contents of IMCL using jMRUI (Java-based Magnetic Resonance User Interface, EU Human Capital and Mobility Networks, France) software.

Lactobacillus reuteri capsules and placebo capsules

Lactobacillus reuteri was encapsuled by Nutraceutix (Nutraceutix, Inc. Redmond, WA, USA). Nutraceutix has a patented technology to encapsulate probiotics with a protective layer, to protect probiotics from gastric acid, while providing an optimal release throughout the intestine (BIO-*tract*® tablets). Probiotics and placebos were produced in optically identical capsules. Packaging and organoleptic appearance was identical, too. All capsules were generously provided by Nutraceutix (Nutraceutix, Inc. Redmond, WA, USA). Each participant received the capsules in the corresponding bottle allocated by an independent researcher with no further involvement in this clinical trial, assigned according to the randomization list. The appropriate package was handed to the particular participant.

Statistical data analysis and power calculation

Statistical data analysis and the power calculation were performed in cooperation with Dr. Klaus Strassburger of the Department of Biometry and Epidemiology at the German Diabetes Center.

Power calculation

As no relevant data preexisted from probiotic studies reporting changes of incretins, particularly of GLP-1 concentration, we assumed data from studies with prebiotics and used this as a basis for power calculation. Group size was calculated in order to detect a

mean difference of one SD before versus after intervention, with a probability (power) of at least 80%. Mean differences between verum and placebo of at least 1.33 SD can be detected with a power of 80%.

Statistical data analysis

SAS for Windows Version 9.2 (SAS Institute, Cary, NC, USA) was used for all analyses. Data are presented as means and standard deviations (mean ± SD) or medians and interquartile range (median [IQR]), as appropriate. Variables with skewed distribution were log transformed before further analysis.

To test differences between treatment arms taking the lean-obese status into account, we first used two-way ANOVA with interaction. In case of a statistically significant interaction term, results were reported separately for the lean and obese group otherwise two-way ANOVA without interaction were used to adjust the treatment effect for the lean/obese status and results were reported for the combined groups.

In the case that variables were not normally distributed before or after log-transformation, differences between treatments were tested by one-way Kruskal–Wallis analysis of variance applied separately to samples of obese and lean subjects.

Pearson's correlation coefficients were used to describe inter-individual (across subjects) correlations between variables and also used to calculate intra-individual (within subject) correlation between variables measured during the OGTT and isoglycemic test. To test whether the mean intra-individual correlations are different from zero, one-sample t-test were performed after normalizing the single intra-individual correlations by applying Fisher's-z-transformation.

P-values from two-sided tests less than or equal to 5% were considered to indicate significant differences.

AUCs of the various compounds are calculated with the trapezoidal rule. Changes in the incretin effect were calculated by AUC of insulin, C-peptide, glucagon, GLP-1 and GLP-2 during OGTT and isoglycemic test. Calculated ΔAUC was defined as AUC during OGTT minus AUC during isoglycemic test. Due to the positive skewness, these AUCs were log-transformed (natural logarithm). Differences of these ΔAUC before and after intervention were calculated.

Results

Recruitment and enrollment of study subjects

Subjects were recruited by newspaper advertisement, flyer, and during an official information event at the German Diabetes Center. More than 130 interested persons contacted us by telephone as resonance to the newspaper advertisement. During the telephone call potential participants got first information about study procedure and duration, allowance, medical examination and measurements.

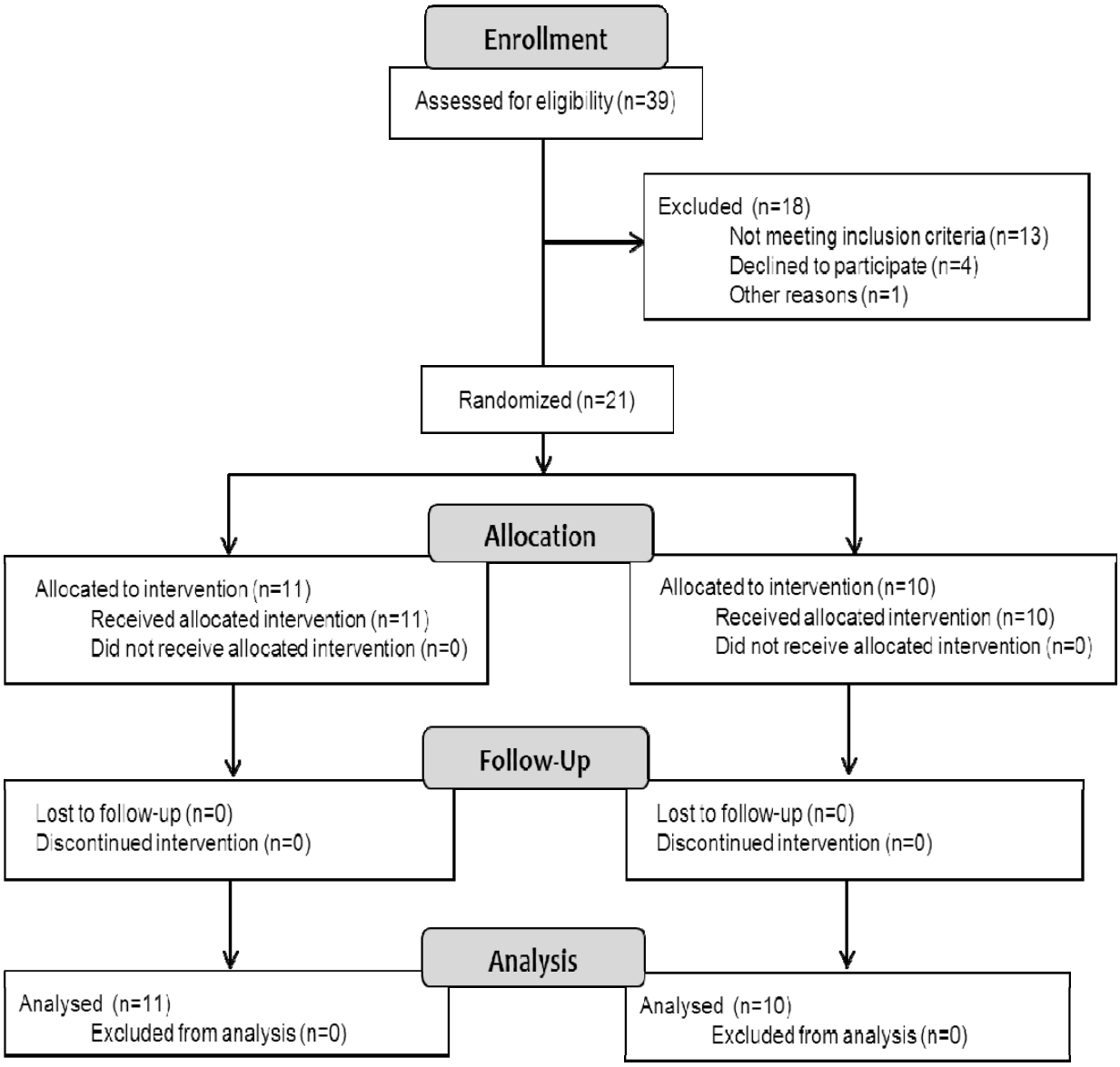

Fig. 11 Enrollment and allocation of participants according CONSORT flow diagram

After written informed consent, 39 subjects were screened for the study throughout October 2011 until August 2012. Of the screened persons 13 subjects did not fullfill the inclusion criteria (e.g. 2 subjects because of antibiotic treatment, 1 subject because of weight changes before study start, 1 subject because of common cold during run-in phase), 4 subjects abstained from the study as they did not want to follow dietary restrictions, 1 person refrained from participating without giving any reasons (**figure 11**).

Table 4 Baseline characteristics of study cohort

	Combined	**Lean**	**Obese**
Subjects [female / male]	11 / 10	6 / 5	5 / 5
Age [years]	50±7	49±7	51±7
BMI [kg/m^2]	29.2±7.0	**23.6±1.7**	**35.5±4.9**
Waist circumfence [cm]	99.1±17.7	**83.3±11.5**	**111.9±9.0**
HbA1c [%]	5.3±0.4	5.2±0.3	5.5±0.4
Fasting BG [mg/dl]	78±7	77±9	79±6
Fasting FFA [µmol/l]	439 [370-631]	439 [246-522]	462 [386-678]
OGIS [ml/min/m^2]	528±92	**569±94**	**482±67**
Adaptation index	0.808 [0.511-0.924]	0.615 [0.509-0.924]	0.830 [0.720-0.957]
Disposition index	3.09 [2.68-5.09]	2.76 [2.33-3.08]	4.77 [4.33-7.55]
Insulinogenic index	179 [123-396]	135 [94-409]	219 [147-395]
hsCRP [mg/dl]§	0.09 [0.05-0.29]	**0.05 [0.04-0.10]**	**0.30 [0.08-0.40]**
Blood pressure sys [mmHg]	138±21	133±23	143±16
Blood pressure dias [mmHg]	85±11	**77±9**	**93±6**
Resting energy expenditure [kcal]	1629±285	**1457±248**	**1819±191**
Gastric emptying [t½]$^{\#}$	84±34	90±36	78±32
Treatment adherence*	21/21	11/11	10/10

Mean ± SD and median [interquartile ranges] are given for normally and log-normally distributed data, respectively. Significant differences ($p<0.05$) between lean and obese subjects are high lightened in bold (unpaired t-test). Resting energy expenditure and hsCRP§ were determined before intervention. The half time (t1/2) of gastric emptying$^{\#}$ was measured using the ^{13}C-octanoic acid breath test. Compliance* was confirmed by tablet counting.

The remaining 21 participating subjects were 11 lean, healthy control subjects (6 females and 5 males) and 10 obese non-diabetic subjects (5 females and 5 males) with a mean

age ± SD of 49±7 years and 51±7 years, respectively (**table 4**). Five of the lean subjects received placebo and 6 received *L. reuteri*. Of the obese subjects, 5 received placebo and 5 received *L. reuteri.*

Baseline characteristics of study cohort

As expected, the obese group had a higher BMI compared to the lean subjects (23.6±1.7 vs. 35.5±4.9 kg/m^2, p<0.001), elevated levels of body fat mass (40±9 vs.28±6 %, p<0.003) and lean body mass (63.5±9.5 vs. 50.7±11.1 kg, p<0.02), but did not differ in regard to concentrations of fasting plasma glucose, glucose tolerance as determined by oral glucose tolerance test (OGTT), gastric emptying, treatment adherence, HbA1c and systolic blood pressure (**table 4**). Insulin sensitivity assessed by OGIS was lower in obese subjects, whereas hsCRP and diastolic blood pressure were higher. Treatment adherence as determined by capsule counting that showed 99.8% compliance in obese and 100% in lean subjects. Likewise, there were no differences of treatment adherence, confirmed by tablet counting between *L. reuteri*- and placebo- groups.

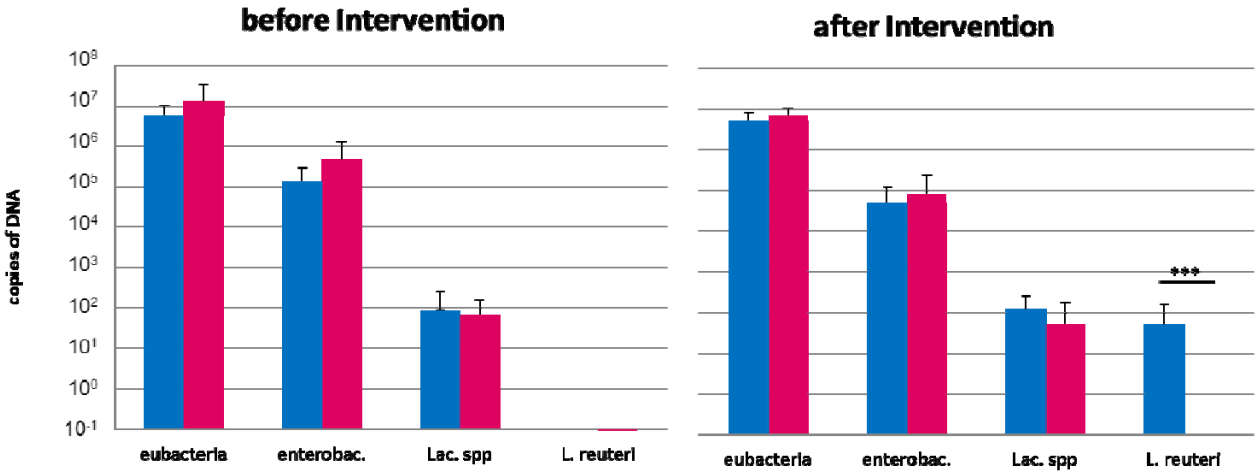

Fig. 12 Abundance of bacterial populations in faecal samples
Total bacterial load (eubacteria), enterobacterial content (enterobac.), and Lactobacillus content (*Lac. spp*), were comparable between intervention- (blue) and placebo-group (red), and did not change upon treatment with *L. reuteri. L. reuteri* content was increased at the end of trial (p<0.0001) and enlarged compared to placebo (***p<0.0001). Abundance of bacterial populations was measured by quantitative real-time PCR. Shown are mean ± SD, Fischer´s exact t-test.

Abundance of bacterial populations in faecal samples

Faecal samples were obtained from all 21 subjects before intervention, and from all but one subject at the end of intervention. From one lean subject of the placebo group we did not receive a sample after intervention. Another lean subject of the placebo group was

tested positive for *L. reuteri* in *L. reuteri* qPCR before intervention (0.249 DNA-copies) and was still positive after intervention (0.041 DNA-copies), but with lower concentration. All other faecal samples were *L. reuteri*-negative before intervention.

Stool samples of the subjects ingesting *L. reuteri* were tested positive upon treatment in *L. reuteri* qPCR, whereas those of the placebo treated subjects remained negative ($p<0.0001$). As shown in **figure 12**, Treatment with neither *L. reuteri* nor placebo lead to an alteration in total bacterial load, enterobacteria or Lac. spp. content. Interestingly, these findings were preserved, and comparable between lean and obese subjects, independent on the group they belong to. Additionally, in the intervention group there were no differences in *L. reuteri* content between lean and obese subjects.

Anthropometry

The anthropometric parameters body weight [kg], BMI [kg/m^2], body fat mass [%], and lean body mass [kg] of the two treatment groups were unaffected by *L. reuteri* or Placebo treatment (**table 5**). Also waist/hip-ratio, blood pressure [mmHg] and pulse [/min] of all subjects remained stable during the study (data not shown).

Table 5 Anthropometric data before and after intervention in the respective groups.

		L. reuteri	**Placebo**
Body weight [kg]	Before	87±23	87±22
	After	87±23	88±22
Body fat [%]	Before	35±7	34±11
	After	33±8	34±11
Lean body mass [kg]	Before	56±13	56±12
	After	58±13	56±12
Intrahepatocellular lipids [%]	Before	1.6 [0.5 - 15.9]	2.4 [1.8 - 14.7]
	After	2.7 [0.6 - 14.9]	1.5 [0.9 - 21.7]
Intramyocellular lipids [%] m. soleus	Before	1.4 [0.9-1.7]	1.5 [0.9 - 2.3]
	After	1.1 [0.6 - 2.2]	1.2 [0.7 - 2.1]
Intramyocellular lipids [%] m. tibialis ant.	Before	0.5 [0.3 - 1.0]	0.8 [0.4 - 0.8]
	After	0.7 [0.6 - 1.0]	0.6 [0.2 - 0.8]

Mean ± SD and median [interquartile ranges] are given for normally and log-normally distributed data, respectively. To test for differences between treatment arms adjusted for body weight, we used two-way ANOVA. Neither before nor after intervention, significant differences in anthropometric data and ectopic fat content between the *L. reuteri* and the placebo group, were found.

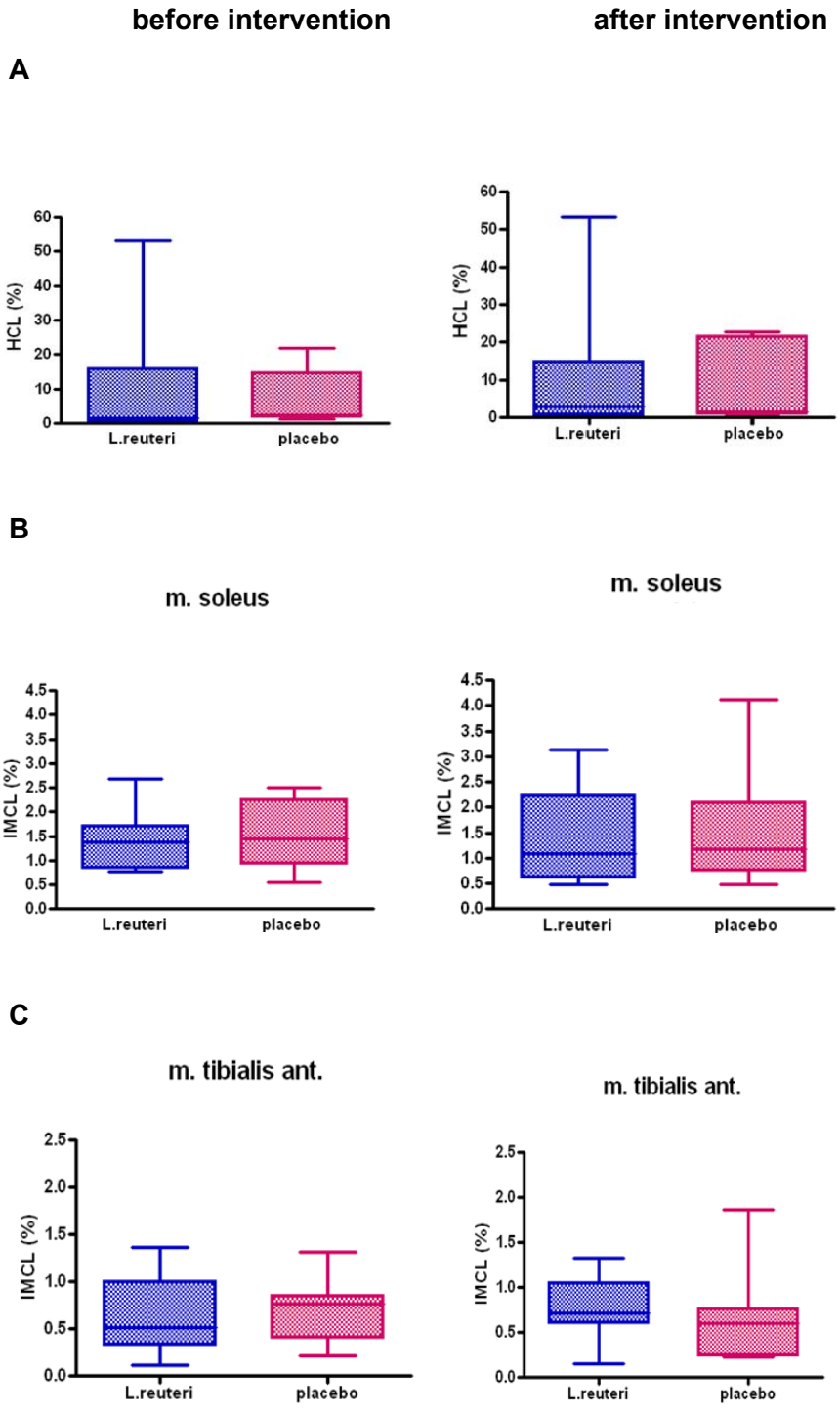

Fig. 13 Ectopic lipid content in *L. reuteri* and placebo treated subjects
L. reuteri (blue) and placebo (red) treated subjects, before and after intervention. Shown are box and whiskers, the line in the box gives median, bars and boxes give interquartile range (IQR), error bars give the minimum and maximum. Intrahepatocellular lipids (HCL) (A), intramyocellular lipids (IMCL) measured in musculus (m.) soleus (B) and m. tibilais anterior (C).

Ectopic lipid content

Ectopic lipid content was assessed in 8 lean (4 *L. reuteri*, 4 placebo) and 7 obese (4 *L. reuteri*, 3 placebo) subjects. Two subjects did not undergo this investigation as they suffered from claustrophobia, one subject had implants with contraindication for MRS, and two did not participate in this assessment for other reasons.

Ingestion of *L. reuteri* did not affect ectopic lipid content measured by MR spectroscopy - neither for intrahepatocellular lipid content (HCL) nor for intramyocellular lipid content (IMCL) determined in the musculus (m.) soleus and m. tibilais anterior (**table 5, figure 13 A-C**). The BMI correlated with the intrahepatocellular lipid content before ($r=0.722$, $p<0.003$) and after intervention ($r=0.625$, $p<0.014$) but not with IMCL in m. soleus and m. tibilais anterior - neither before nor after intervention.

Tissue-specific whole body and hepatic insulin sensitivity

As expected, lean subjects showed better (higher) whole-body insulin sensitivity compared to obese subjects before (M-Value 9.6 [IQR 6.4 – 11.4] vs. 5.4 [3.7 – 7.9] mg/kg*min, $p<0.05$) and after intervention (M-Value 8.5 [7.4 - 10.3] vs. 7.0 [4.0 - 8.3] mg/kg*min, $p<0.05$, **figure 14 A**). The comparison of whole-body insulin sensitivity between *L. reuteri*- and placebo-group neither showed differences before (M-Value 8.2 [5.7 - 10.0] vs. 6.4 [4.4 - 11.1]) nor after intervention (M-Value 7.4 [6.5 - 11.5] vs. 8.0 [6.6 - 9.1]). Likewise, intervention did not alter whole body insulin sensitivity.

L. reuteri neither affected endogenous glucose production (EGP) at baseline (1.9±0.4 vs. 1.6±0.3 mg/kg*min, data not shown) nor insulin-stimulated EGP suppression. EGP was also comparable between *L. reuteri*- and placebo-group before and after intervention (**figure14 B**).

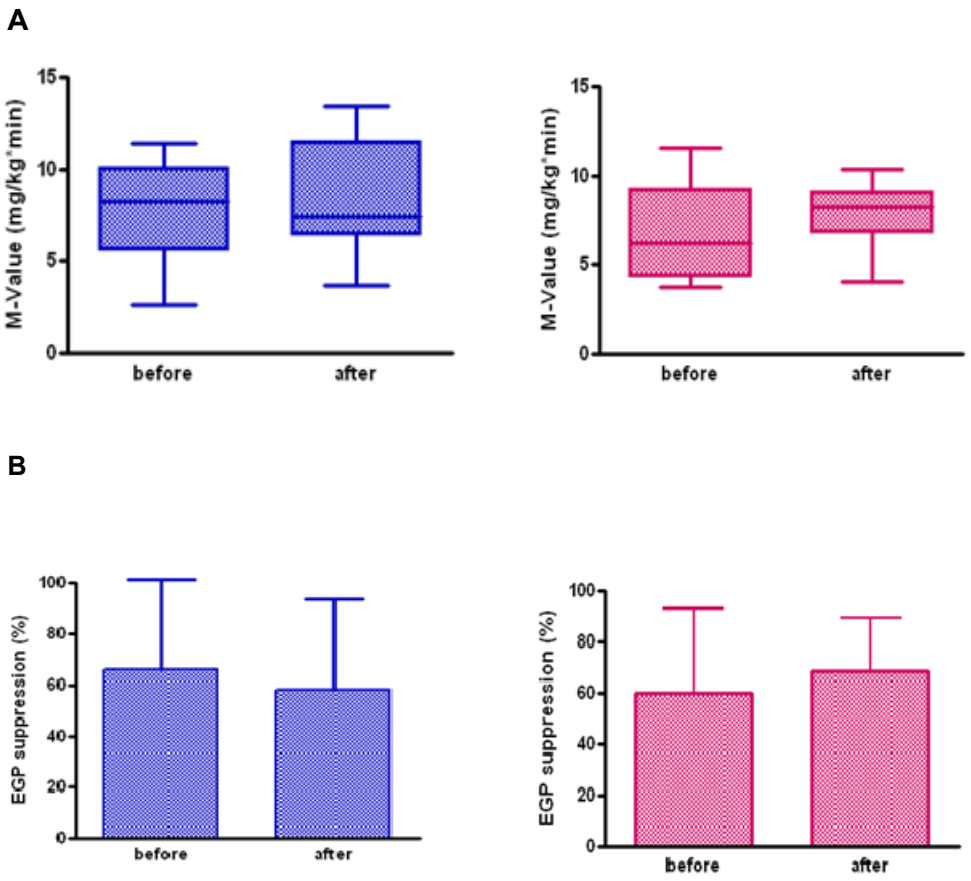

Fig. 14 Results from the hyperinsulinemic-euglycemic clamp before and after intervention in *L. reuteri* (blue), and placebo (red) treated groups. M-Value represents the whole body insulin sensitivity (A). Endogenous glucose production (EGP) represents the hepatic insulin sensitivity (B). Given are box and whiskers, the line in the box gives median, bars and boxes give interquartile range (IQR), error bars give the minimum and maximum.

Energy expenditure and substrate oxidation

The resting energy expenditure (REE) measured in kcal at baseline and during clamp differed between lean and obese subjects (**table 4**) and did not change during the study. In line with the results from hyperinsulinemic-euglycemic clamp, the substrate oxidation, especially the oxidative and non-oxidative glucose utilization, which both increased during clamp, showed neither differences between groups nor towards intervention (**figure 15 A**). Lipid oxidation rates during hyperinsulinemia were reduced by *L. reuteri* 94% and by placebo 102% compared to baseline, without any differences between both groups (**figure 15 B**).

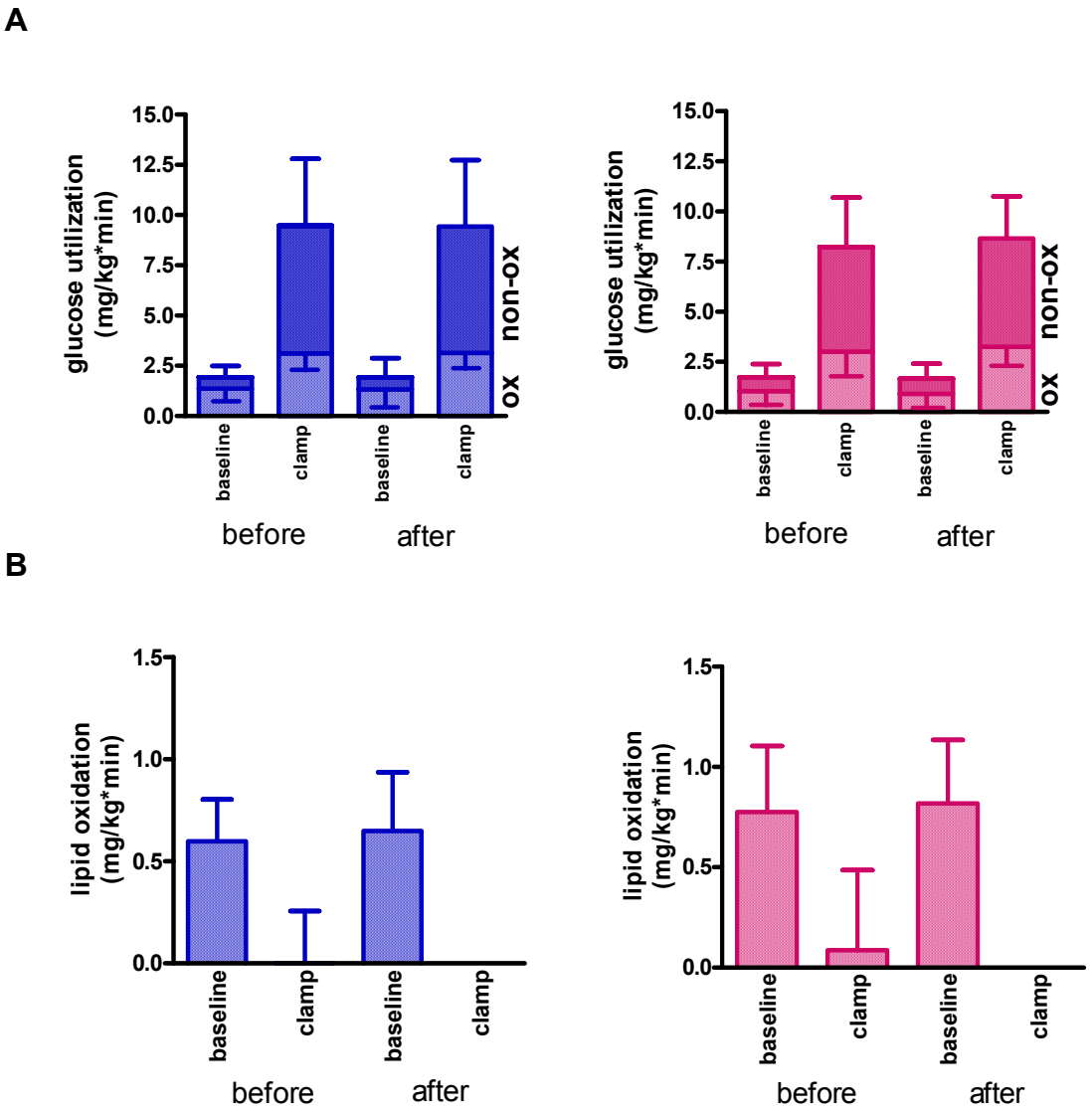

Fig. 15 Lipid oxidation and glucose utilization before and after intervention
Bars show the glucose utilization (A) and lipid oxidation rates (B) in *L. reuteri* (blue) and placebo (red) treated groups, respectively. Lipid oxidation and glucose utilization were assessed at baseline and during steady state conditions of hyperinsulinemic-euglycemic clamp. Hatched bars show the non-oxidative glucose utilization (non-ox), checkered bars show oxidative glucose utilization (ox). Mean and SD are given before and after intervention.

Glucose tolerance

Blood glucose concentrations measured from OGTT and isoglycemic i.v. glucose infusion test (infusion of 20% glucose solution) remained unchanged upon treatment (**figure 16**). Likewise, the total glucose infusion rate calculated in total g glucose showed no differences upon treatment (before 26.2 [14.5 - 43.6] vs. after 29.7 [16.0 – 35.0]). Glucose infusion rates of lean and obese subjects differed after (13.0 [6.9 - 29.6] vs. 22.0 [37.5 –

47.9], p<0.004), but not before intervention (23.2 [10.3 – 26.2] vs. 22.3 [19.3 – 51.3], p=0.3073).

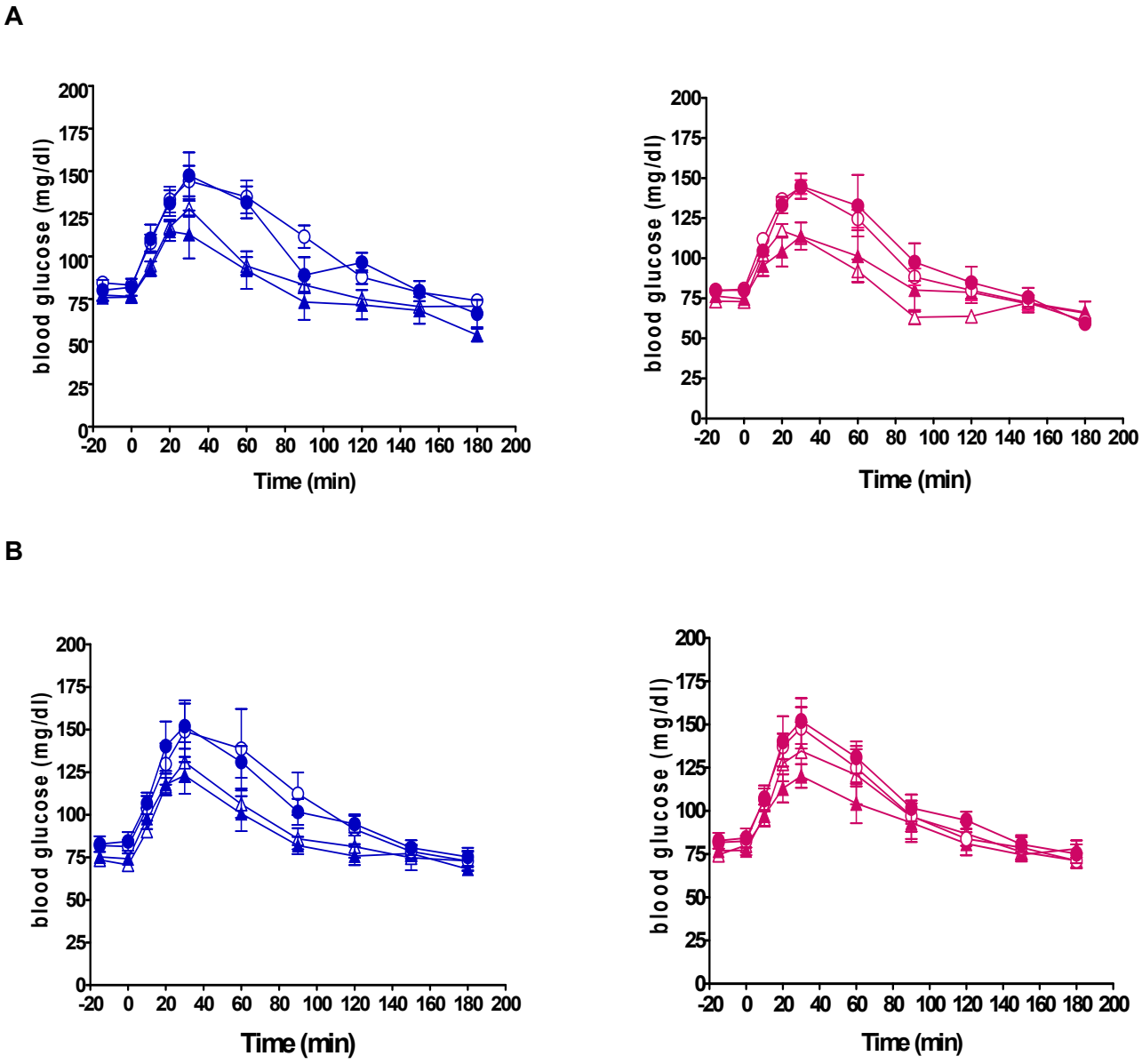

Fig. 16 Blood glucose concentrations during OGTT and isoglycemic i.v. glucose infusion
OGTT (A) and isoglycemic i.v. glucose infusion (B) before and after Intervention of *L. reuteri*- (blue) and placebo-group (red), respectively. Shown are data from lean (triangles) and obese subjects (circles) before (open symbols) and after intervention (closed symbols) as mean and SEM. *p<0.05 vs. baseline.

Insulin secretion and incretin effect

Intravenous and oral glucose-stimulated C-peptide and insulin levels were determined to assess β-cell function. Insulin and C-peptide secretion were stimulated to a higher extend by orally than by intravenously applied glucose in all groups ($p<0.01$) as expected.

After Intervention, insulin secretion was increased 20 min and 60 min after glucose application, in parallel with increased C-peptide levels at these time points, while glucagon remained unaltered. The increased concentrations of insulin and C-peptide were detectable in lean and obese subjects (**figure 17 A-B**). Fasting concentrations of glucagon differed between lean and obese subjects before ($p<0.05$) but not after intervention ($p=0.08$). By trend glucagon concentrations were higher in obese compared to lean subjects (**figure 17 C**).

Comparison of the relative difference of ΔAUC before intervention versus ΔAUC after intervention, to assess the incretin effect, showed that ΔAUC of insulin and C-peptide were increased in the *L. reuteri*-group at the end of the study ($p<0.05$), but not in the placebo group (**figure 18 A-B**). When we determined single time points of insulin and C-peptide during oral glucose stimulation, the higher insulin and C-peptide concentrations in the *L. reuteri* group at the end of the trial was seen with significantly increased concentrations at 20 and 60 min of glucose stimulation ($p<0.05$, **figure 17 A-B**). The maximal response (peak at 60 min) for insulin and C-peptide was increased at the end of the study in the intervention group ($p<0.016$, $p<0.018$, respectively). Concentrations of glucagon remained stable throughout the study and were unaffected by intervention (**figure 18 C**). There were no glucose-stimulated alterations and no maximal responses (peak) detectable for glucagon. Glucose-stimulated concentrations of glucagon showed no differences between lean and obese subjects (**figure 17 C**).

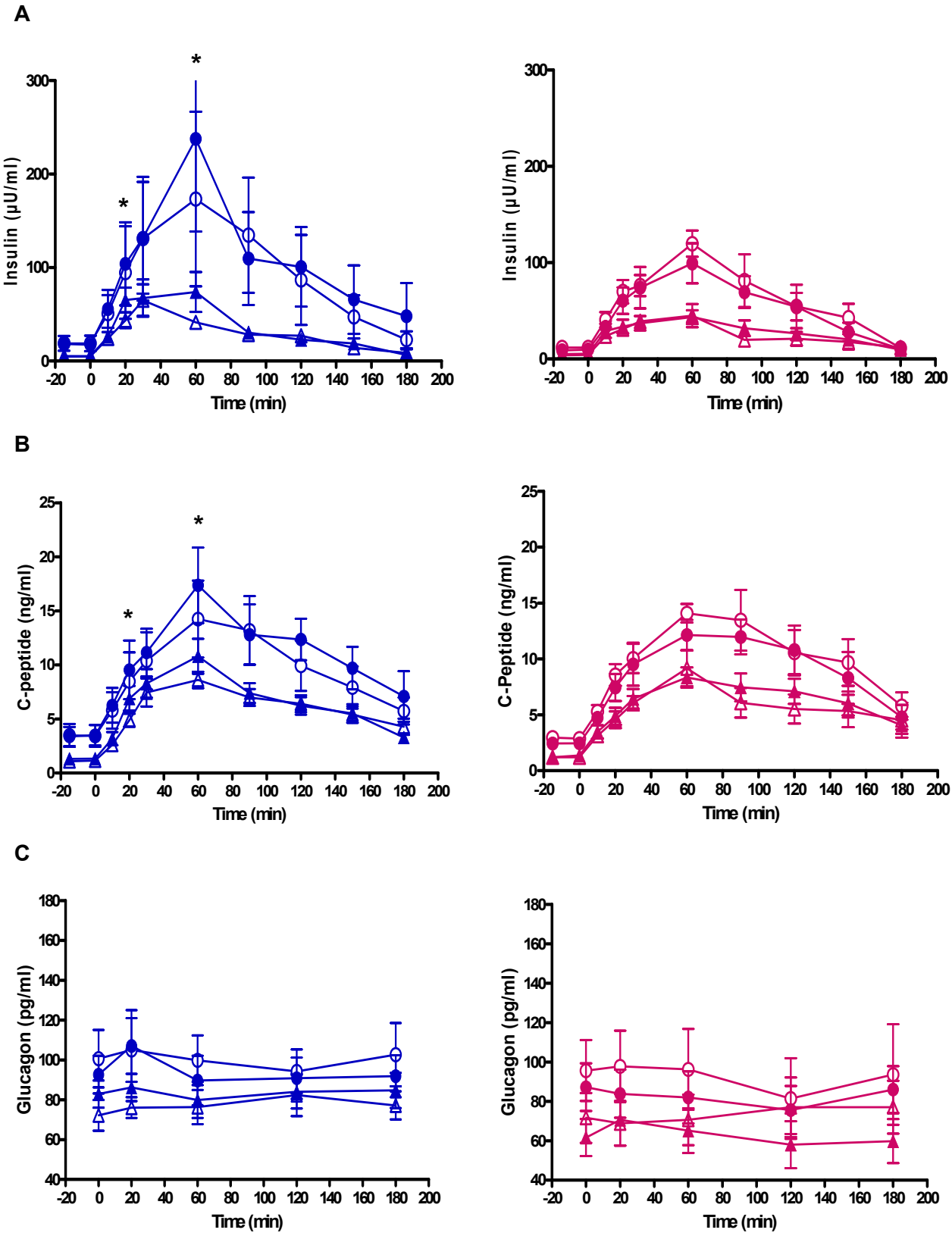

Fig. 17 Concentrations of insulin, C-peptide and glucagon during OGTT
Comparison of insulin (A), C-peptide (B) and glucagon (C) during OGTT before versus after intervention of *L. reuteri*- (blue) and placebo-group (red), respectively. Shown are data from lean (triangles) and obese (circles) subjects before (open symbols) and after intervention (closed symbols). Mean and SEM, *p<0.05 before vs. after intervention.

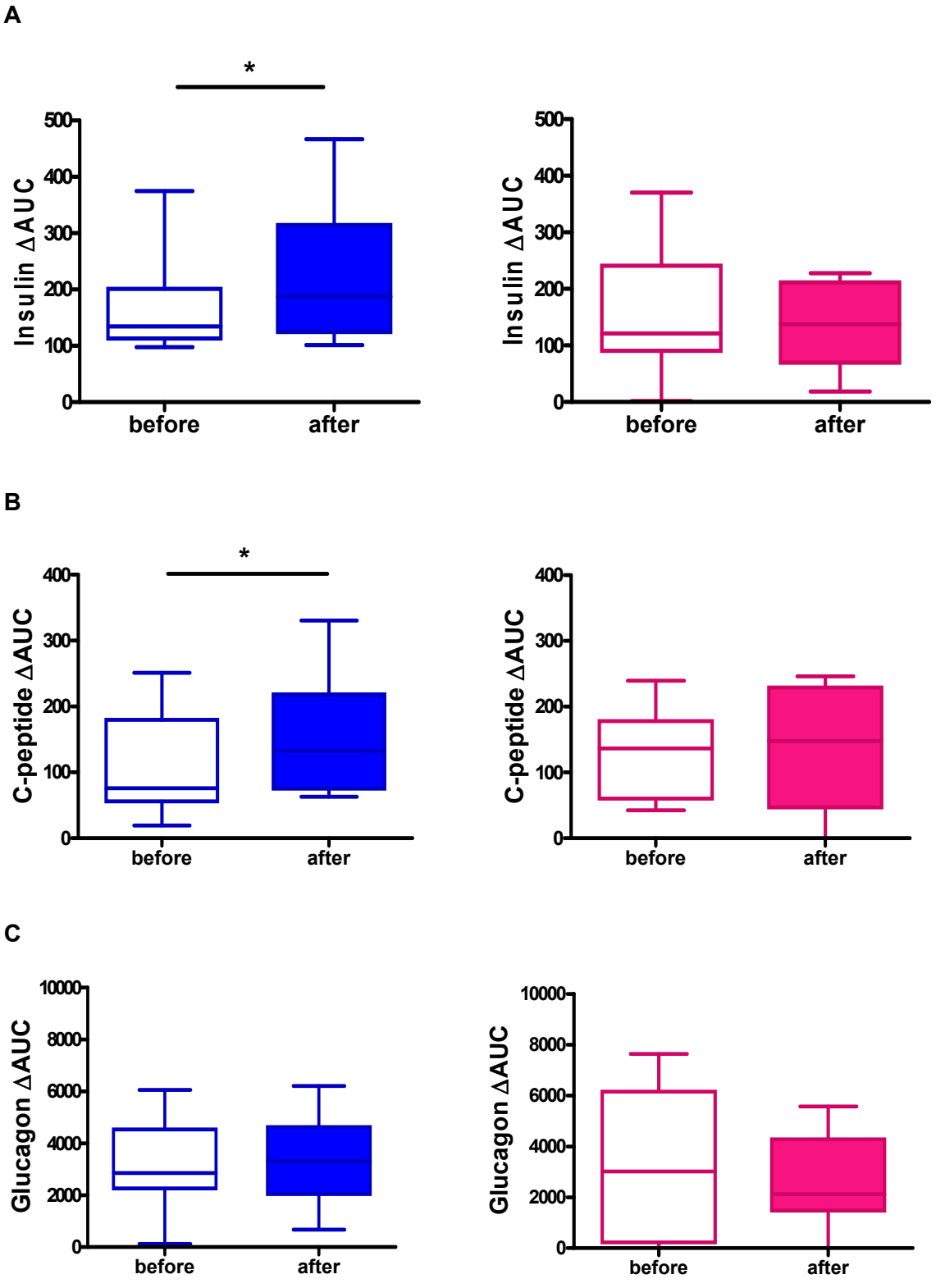

Fig. 18 ΔAUC of Insulin, C-peptide and glucagon
Comparison of Δ area under the curve (AUC) of insulin (A), C-peptide (B) and glucagon (C) before versus after intervention of *L. reuteri*- (blue) and placebo-group (red), respectively. Shown are ΔAUC, calculated as delta of AUC of OGTT and AUC of the isoglycemic test, before (open symbols) and after intervention (closed symbols). Mean and SEM, *p<0.05 ΔAUC before vs. ΔAUC after intervention.

Indices of β-cell function calculated by validated mathematical model analyses taking glucose, insulin and C-peptide values of OGTT into account, the adaptation index, the disposition index, and the insulinogenic index, were increased in the *L. reuteri*-group ($p < 0.05$) after treatment and altered when compared to the corresponding indices of the placebo-group, which remained unchanged (**table 6**).

Table 6 Changes of indices of β-cell function upon intervention

		L. reuteri	**Placebo**
OGIS	before	476±95	533±100
	after	521±128	527±74
Adaptation index	before	0.578 [0.480- 0.835]	0.765 [0.489 - 0.947]
	after	**0.793 [0.606 - 0.874]***	**0.728 [0.505 - 0.822]**
Disposition index	before	3.05 [2.21 - 4.28]	3.89 [2.24 - 5.78]
	after	**3.74 [2.85 - 7.97]***	**2.69 [2.24 - 4.59]**
Insulinogenic index	before	153 [143 – 660]	551 [252 – 714]
	after	**264 [131 – 307]**	**261 [156 – 472]***

Mean ± SD and median [interquartile range] are given for normal and log-normal distributed data, respectively. Significant differences (before/after intervention) of *L. reuteri* vs. placebo are highlighted in bold, *significant difference before/after intervention within the treatment group, $p<0.05$. OGIS is an index for insulin sensitivity, while the adaptation index, the disposition index, and the insulinogenic index are indices of β-cell function taking insulin sensitivity into account.

Incretin secretion

The fasting plasma concentrations of GLP-1, GLP-2 and GIP were in a range as expected. The concentrations after intervention were median 7.0 (5.0-8.0) pmol/l for GLP-1, 8.0 (8.0-10.0) pmol/l for GLP-2 and mean 62.3±35.5 pg/ml for GIP and did not change when compared to baseline levels except for GLP-1 (**figure 19 A-C**). During the OGTT we observed an increase in plasma concentrations for all three gut hormones with a maximum seen at 30 min for GLP-1, 60 min for GLP-2 and GIP (**figure 19 A-C**), before and after intervention. The maximal response (peak) for GLP-1 and GLP-2 was increased at the end of the study in the intervention group for lean subjects ($p<0.02$ and $p<0.04$, respectively) but not for obese subjects. The maximal response (peak) for GLP-1 differed in the *L. reuteri* group after intervention compared to placebo ($p<0.04$, **figure 19**).

After treatment fasting plasma GLP-1 levels were increased within the intervention group ($p<0.02$, min 0), and were by trend increased ($p=0.052$, min 0) when compared to the placebo group. Glucose stimulated GLP-1 levels were higher after treatment at minute 20 ($p<0.03$), 30 ($p<0.01$), 60 ($p<0.01$), 120 ($p<0.05$), and 180 ($p<0.03$) of the OGTT within the *L. reuteri* group (**figure 19 A**), and increased compared to placebo at minute 20 ($p<0.01$), 30 ($p<0.01$), 60 ($p<0.01$), 120 ($p<0.05$), and 180 ($p<0.03$) of the OGTT. Likewise, plasma GLP-2 levels were by trend increased after treatment at minute 20 ($p<0.057$) and 60 ($p<0.065$), within the *L. reuteri* group (**figure 19 B**), and increased compared to placebo at minute 20 ($p<0.05$) and 60 ($p<0.02$). Glucose stimulated GLP-1 and GLP-2 levels, measured at several time points during OGTT were increased after intervention.

As GLP-1 and GLP-2 are co-secreted, we investigated their correlation. Plasma GLP-1 and GLP-2 concentrations from OGTT samples showed a similar pattern and correlated positively intra-individually before ($r=0.86$; $p<0.0001$) and after intervention ($r=0.82$; $p<0.0001$). Likewise, AUCs of GLP-1 and GLP-2 correlated with each other before ($r= 0.87$, $p<0.0001$) and after ($r= 0.95$, $p<0.0001$) intervention. Further, GLP-1 and GLP-2 correlated intra-individually with insulin ($r=0.74$ $p<0.0001$ and $r=0.76$ $p<0.0001$ respectively) and C-peptide concentrations ($r=0.79$ $p<0.0001$ and $r=0.72$ $p<0.0001$ after intervention respectively) during OGTT but not during isoglycemic i.v. glucose infusion test (data not shown).

Glucose stimulated AUC of GLP-1 (during OGTT) was increased in the *L. reuteri* group after intervention ($p<0.003$) and elevated when compared to the placebo group (**figure 19 A**). Similarly, glucose stimulated AUC of GLP-2 was increased in the intervention group compared to placebo ($p<0.029$, **figure 19 B**). GIP remained unchanged in the intervention group and compared to placebo (**figure 19 C**).

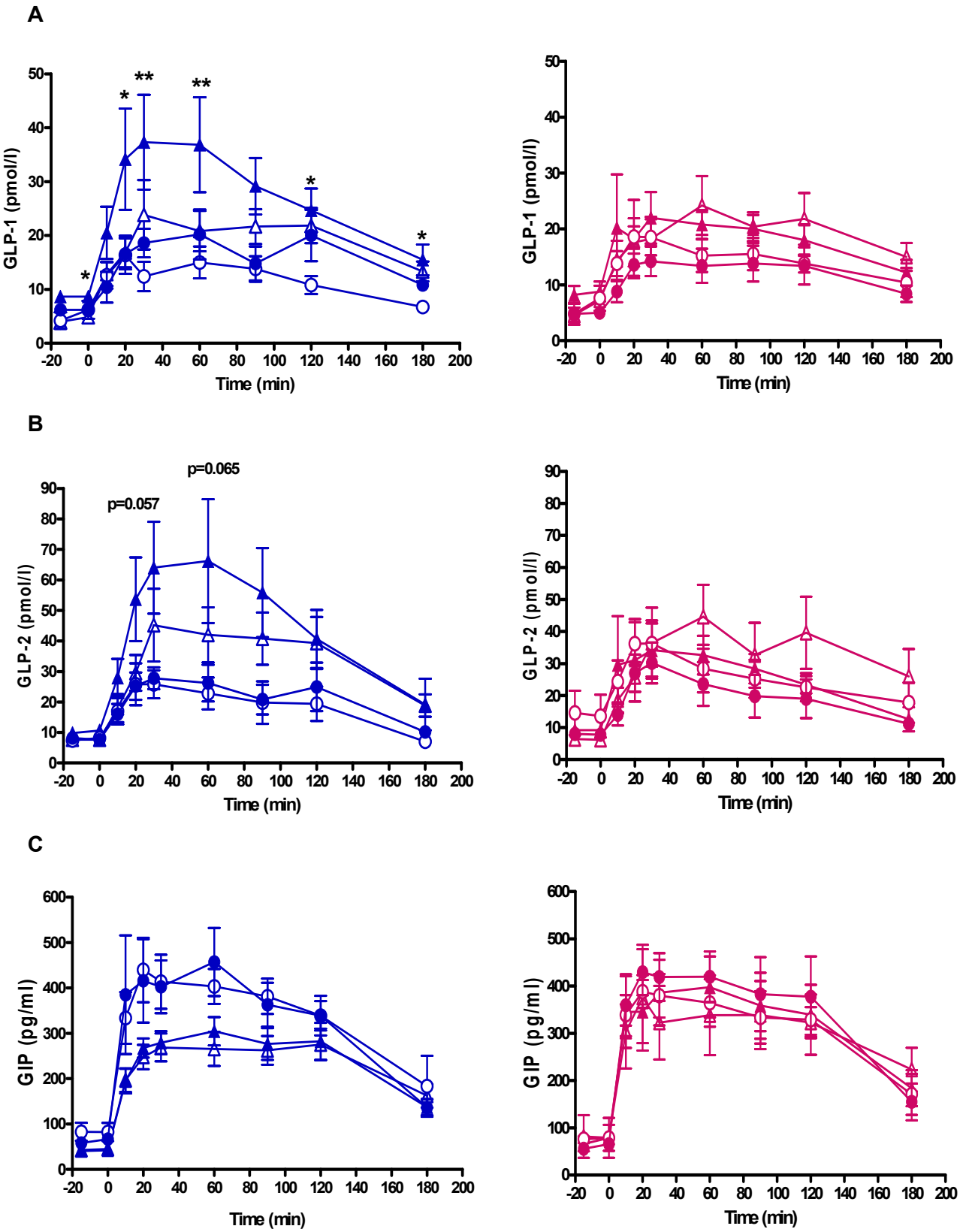

Fig. 19 Gut peptides secretion during OGTT

Gut peptides concentrations during OGTT before versus after intervention in the *L. reuteri*- (blue) and placebo-group (red), respectively. Shown are data from lean (triangles) and obese (circles) subjects before (open symbols) and after intervention (closed symbols) as mean and SEM. Glucagon-like peptide (GLP)-1 (A) and GLP-2 (B), glucose-dependent insulinotropic peptide (GIP) (C), *p<0.05 before vs. after intervention.

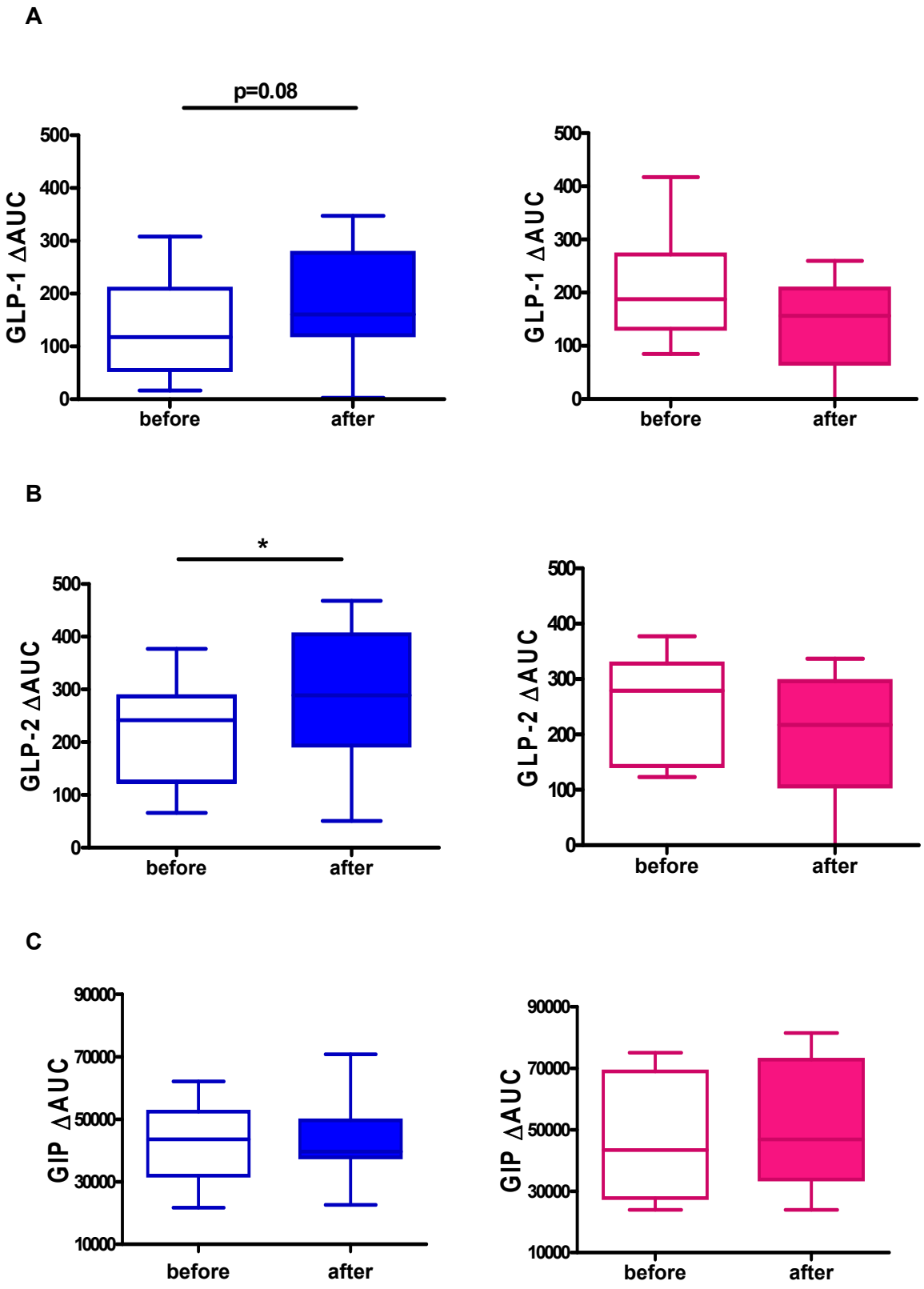

Fig. 20 ΔAUC of glucagon like peptides and glucose-dependent insulinotropic peptide
Comparison Δ area under the curve (AUC) of glucagon like pepdide (GLP)-1 (A), GLP-2 (B) and glucose-dependent insulinotropic peptide (GIP) (C) before versus after intervention of *L. reuteri*- (blue) and placebo-group (red), respectively. Shown are ΔAUC, calculated as delta of AUC of OGTT and AUC of isoglycemic test, before (open symbols) and after intervention (closed symbols). Mean and SEM, *p<0.05 ΔAUC before vs. ΔAUC after intervention.

The ΔAUC of GLP-1 calculated as '*ΔAUC after - ΔAUC before'* to assess the incretin effect, was increased at the end of the study compared to placebo (p<0.004) and tends to be increased after intervention within the *L. reuteri*-group (p=0.087) (**figure 20 A**). The ΔAUC of GLP-2 was increased after intervention in the *L. reuteri*-group (p< 0.02, **figure 20 B**) and significantly increased when compared to the placebo-group (p<0.004). ΔAUC of GIP remained unchanged in both groups (**figure 20 C**). REE before (-0.74, p<0.01; -0.63, p<0.01) and after intervention (-0.64, p<0.01; -0.67, p<0.001) as well as body weight before (-0.63, p<0.05; -0.52, p<0.01) and after (-0.75, p<0.001; -0.75, p<0.001) intervention showed an inverse inter-individual correlation with the AUCs of GLP-1 and GLP-2.

Systemic Inflammation

Obese non-diabetic subjects had higher concentrations in the fasting hsCRP (**figure 21**; p<0.004 and p<0.002) and glucose-stimulated state, during OGTT (p<0.003 and p<0.002), similar to pro- and anti-inflammatory cytokines (IL-1ra, MCP-1, TNF-alpha, **table 7**) before and after intervention. These cytokine concentrations were in an expected range. There were no differences between lean and obese subjects for pro-inflammatory IL-8 and MIP-1β levels, and for reactive oxygen species (TBARS) before, or after intervention (data not shown). Surprisingly, more than 80% of the IFN-γ, IL-6, IL-1β, and IL-10 measurements yielded values below the detection limit or were extrapolated from the standard curve and were therefore not analysed in this study. Upon ingestion of *L. reuteri,* all analysed pro- and anti-inflammatory immune mediators measured remained stable. So did the ratios between analysed pro- and anti-inflammatory immune mediators, including the TNF-α/IL-1ra ratio (**table 7**).

Circulating endotoxin levels

Fasting circulating endotoxin concentrations, as an additional parameter to assess the inflammatory status, also remained stable upon intervention in placebo and *L. reuteri* treated subjects. Interestingly, fasting endotoxin levels were higher in obese subjects before intervention compared to lean subjects (p<0.019) but did not differ between lean and obese subjects after intervention (p=0.169, **figure 22**). Similarly, the measured fasting endotoxin levels correlated with BMI before (r=0.443, p<0.045) but not after intervention (r=0.354, p=0.115). In contrast, fasting hsCRP levels correlated with the BMI before and after intervention (r=0.790, p<0.0001 and r=0.775, p<0.0001, respectively).

Glucose-stimulated endotoxin levels at minute 120 during OGTT showed no significant changes upon intervention, neither in lean nor in obese subjects. But similar to the fasting

levels, glucose-stimulated endotoxin levels differed between lean and obese subjects before ($p<0.049$) but not after intervention (data not shown) and correlated with BMI before ($r=0.503$, $p<0.020$) but not after intervention ($r=0.379$, $p=0.089$).

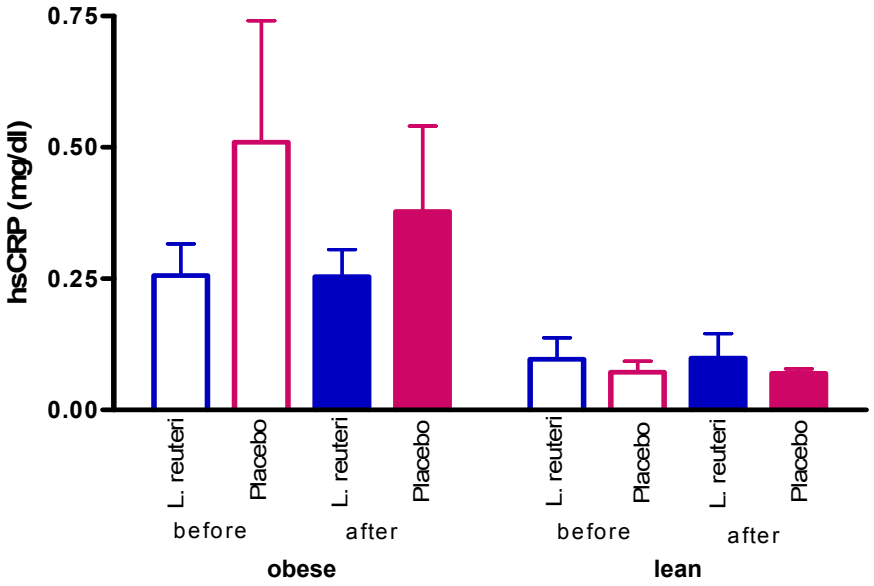

Fig. 21 Fasting hsCRP concentrations
Comparison of fasting hsCRP concentrations of the *L. reuteri* (blue) and the placebo-group (red), before (open) and after intervention (closed bars) in lean and obese subjects. Shown are mean and SEM.

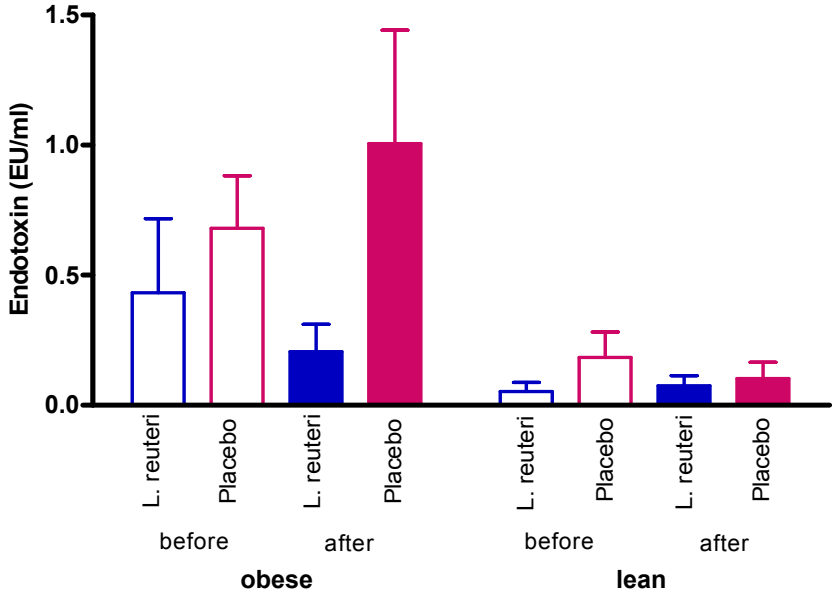

Fig. 22 Fasting endotoxin concentrations
Comparison of fasting endotoxin (lipopolysaccharide, LPS) concentrations of the *L. reuteri*- (blue) and the placebo-group (red), before (open) and after intervention (closed bars) in lean and obese subjects. Shown are mean and SEM.

Circulating adipokine concentrations

Ingestion of *L. reuteri* did not affect serum concentrations of leptin or adiponectin, as markers secreted by the adipose tissue. Fasting leptin was significantly higher in obese compared to lean subjects before ($p<0.05$) and after intervention ($p<0.05$). Subgroup analyses showed no differences based on the intervention neither in obese nor in lean subjects (**figure 23**). The glucose-stimulated leptin concentrations showed similar results (data not shown).

Leptin concentrations correlated positively with LPS ($r=0.512$, $p<0.036$) in lean and obese subjects, and this association persisted after adjustment for BMI ($r=0.512$, $p<0.036$). Further, intra-individual leptin correlated inversely with intestinotrophic GLP-2 ($r=-0.267$; $p<0.028$) and positively with the pro-inflammatory cytokines MIP-1β ($r=0.464$, $p<0.001$) and TNF-α ($r=0.287$, $p<0.033$). Whereas leptin correlated inversely with peripheral insulin sensitivity ($r=-0.433$, $p<0.05$), significance disappeared after adjustment for BMI ($r=0.462$, $p=0.221$).

Fasting adiponectin levels remained stable upon intervention and did not differ in subgroups (**figure 24**), as well as glucose-stimulated adiponectin levels, which showed no differences, neither in lean nor in obese subjects (data not shown).

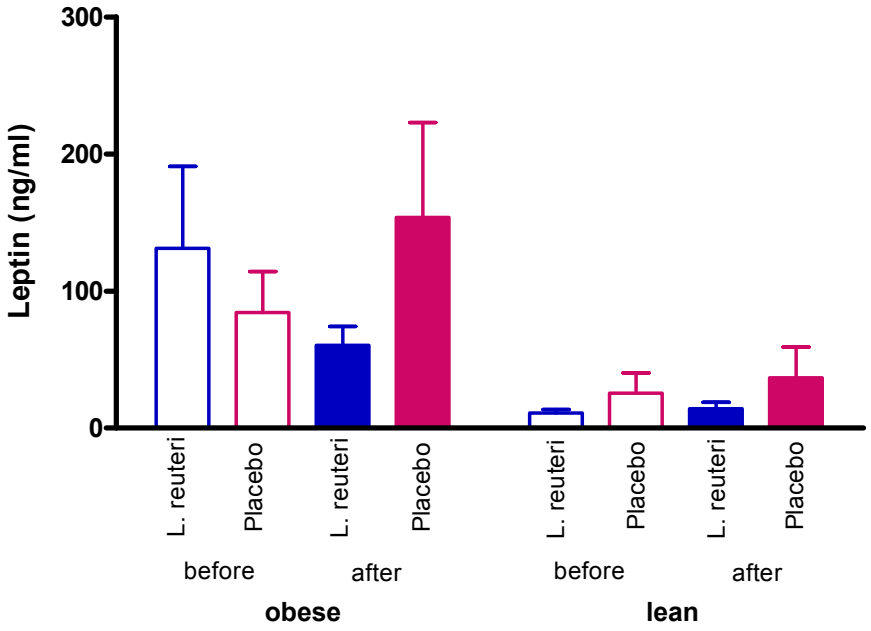

Fig. 23 Fasting leptin concentrations
Comparison of fasting leptin concentrations of the *L. reuteri*- (blue) and the placebo-group (red), before (open) and after intervention (closed bars) differentiated between lean and obese. Shown are mean and SEM.

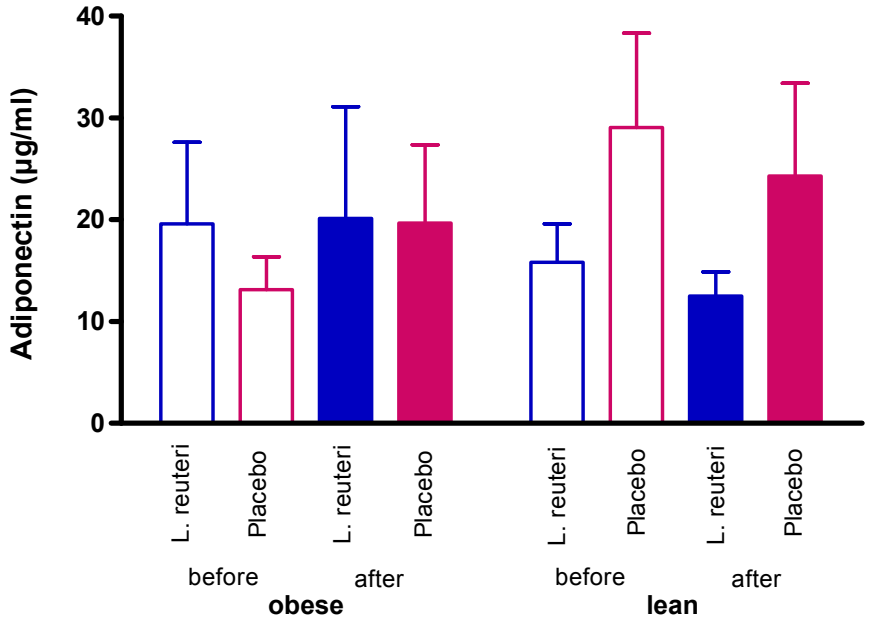

Fig. 24 Fasting adiponectin concentrations
Comparison of fasting adiponectin concentrations of the *L. reuteri*- (blue) and the placebo-group (red), before (open) and after intervention (closed bars) differentiated between lean and obese. Shown are mean and SEM.

Table 7 Systemic inflammatory mediators

		L. reuteri		**Placebo**	
		Lean	**Obese**	**Lean**	**Obese**
hsCRP (mg/dl)	before	0.065 [0.030 - 0.195]	0.290 [0.120 – 0.375]	0.050 [0.040 – 0.115]	0.310 [0.075 – 1.045]
	after	0.065 [0.030 – 0.200]	0.280 [0.150 – 0.345]	0.070 [0.055 – 0.085]	0.210 [0.095 – 0.745]
IL-1ra (pg/ml)	before	643.8 [572.1 – 813.9]	1714.0 [1217.0- 2003.7]	736.7 [570.8 - 874.4]	1270.6 [801.8 – 2543.1]
	after	544.8 [510.3 – 1010.7]	1728.7 [1451.4 – 2615.9]	651.8 [617.7 - 693.9]	1402.7 [929.4 – 2764.4]
MCP-1 (pg/ml)	before	242.5 [211.3 – 280.6]	339.5 [252.2 – 506.1]	326.6 [212.5 - 385.9]	544.8 [507.5 – 565.4]
	after	255.7 [201.0 – 438.1]	297.0 [210.6 – 558.7]	253.0 [222.6 - 280.9]	553.1 [391.0 – 651.1]
TNF-α (pg/ml)	before	3.47 [2.77 – 5.70]	10.90 [2.98 – 65.55]	4.16 [2.53 - 6.80]	30.56 [6.48 – 54.68]
	after	4.48 [2.97 – 20.29]	6.32 [4.05 – 58.80]	2.68 [2.09 - 4.17]	29.50 [6.48 – 45.13]
MIP-1β (pg/ml)	before	71.51 [51.29 – 121.11]	232.02 [96.92 – 300.83]	70.88 [66.69 – 74.49]	154.19 [112.82 – 457.22]
	after	63.15 [42.15 – 136.14]	172.64 [87.41 – 208.48]	68.79 [63.78 – 99.37]	135.60 [113.53 – 473.01]
TNF-α/IL-1ra ratio	before	0.006 [0.004 – 0.013]	0.006 [0.003 – 0.036]	0.008 [0.003 -0.015]	0.010 [0.008 – 0.023]
	after	0.009 [0.005 – 0.024]	0.004 [0.003 – 0.032]	0.004 [0.003 – 0.016]	0.009 [0.008 – 0.019]

Serum concentrations of inflammatory markers are given as median [interquartile range], for the log-normal distributed data. To test for differences between treatment arms adjusted for body weight, we used two-way ANOVA. No significant changes in systemic inflammatory mediators between *L. reuteri* and placebo group neither before nor after intervention were found.

Concentrations of free fatty acids and triglycerides

Free fatty acid (FFA) and triglyceride (TG) concentrations differed between lean and obese subjects ($p<0.05$) and remained unchanged upon intervention. As expected FFA concentrations were significantly supressed during OGTT ($p<0.01$, **figure 25 A**), while TG remained stable (**figure 25 B**).

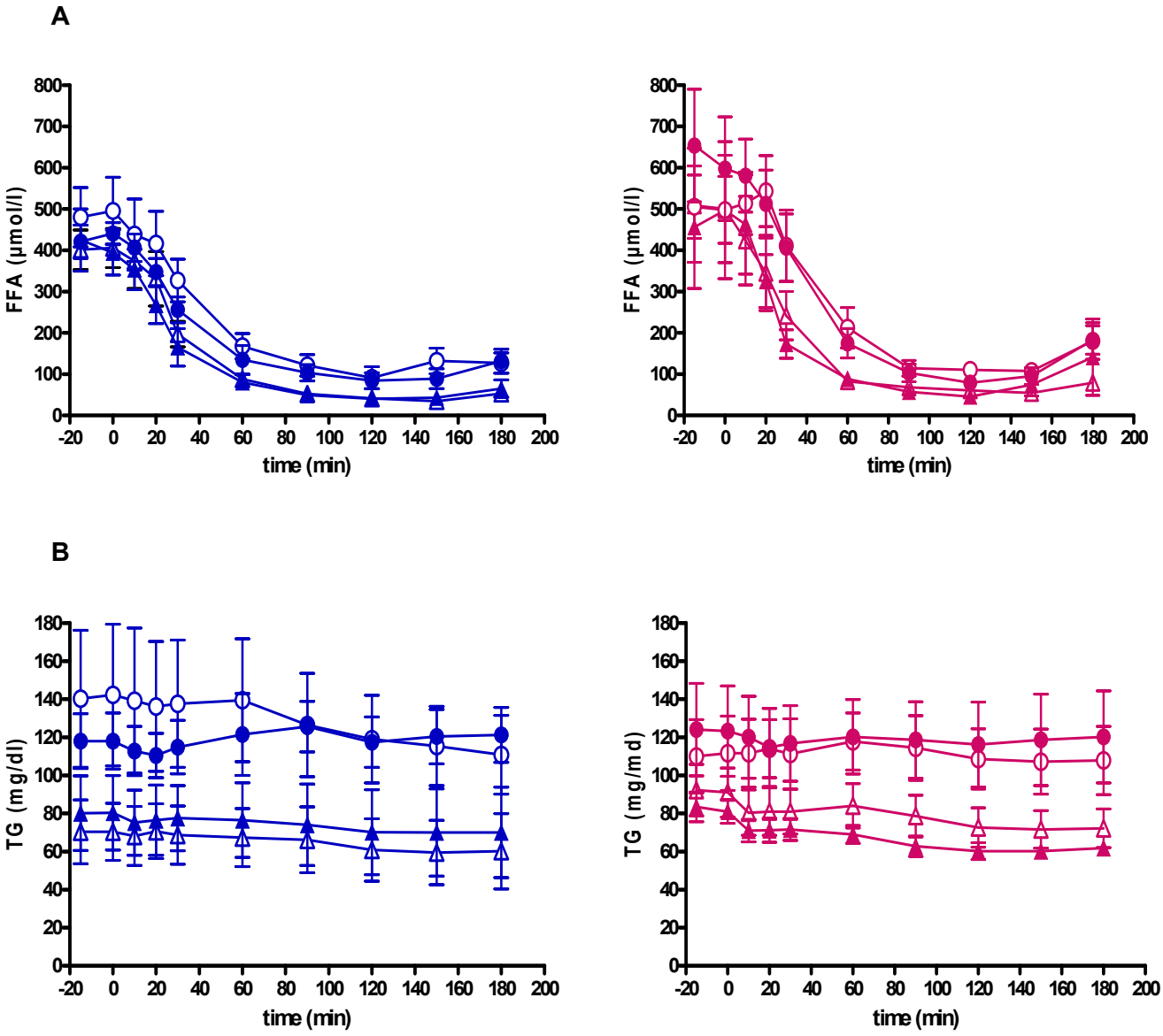

Fig. 25 Free fatty acids (FFA) and triglycerides (TG) during OGTT
Comparison of free fatty acids (FFA) (A) and triglycerides (TG) (B) during OGTT, before versus after intervention of *L. reuteri*- (blue) and placebo-group (red), respectively. Shown are data from lean (triangles) and obese (circles) subjects before (open symbols) and after intervention (closed symbols). Shown are median and SEM, * $p<0.05$ before vs. after intervention.

Discussion

This is the first study demonstrating that ingestion of *L. reuteri* over 4 weeks modulate the secretion of insulin, C-peptide, and the gut hormones GLP-1 and GLP-2, in glucose tolerant lean and obese humans. In contrast, peripheral and hepatic insulin sensitivity and associated parameters like body mass, ectopic fat content, and the levels of systemic inflammatory mediators, adipokines, endotoxin and oxidative stress remained unchanged (**figure 26**).

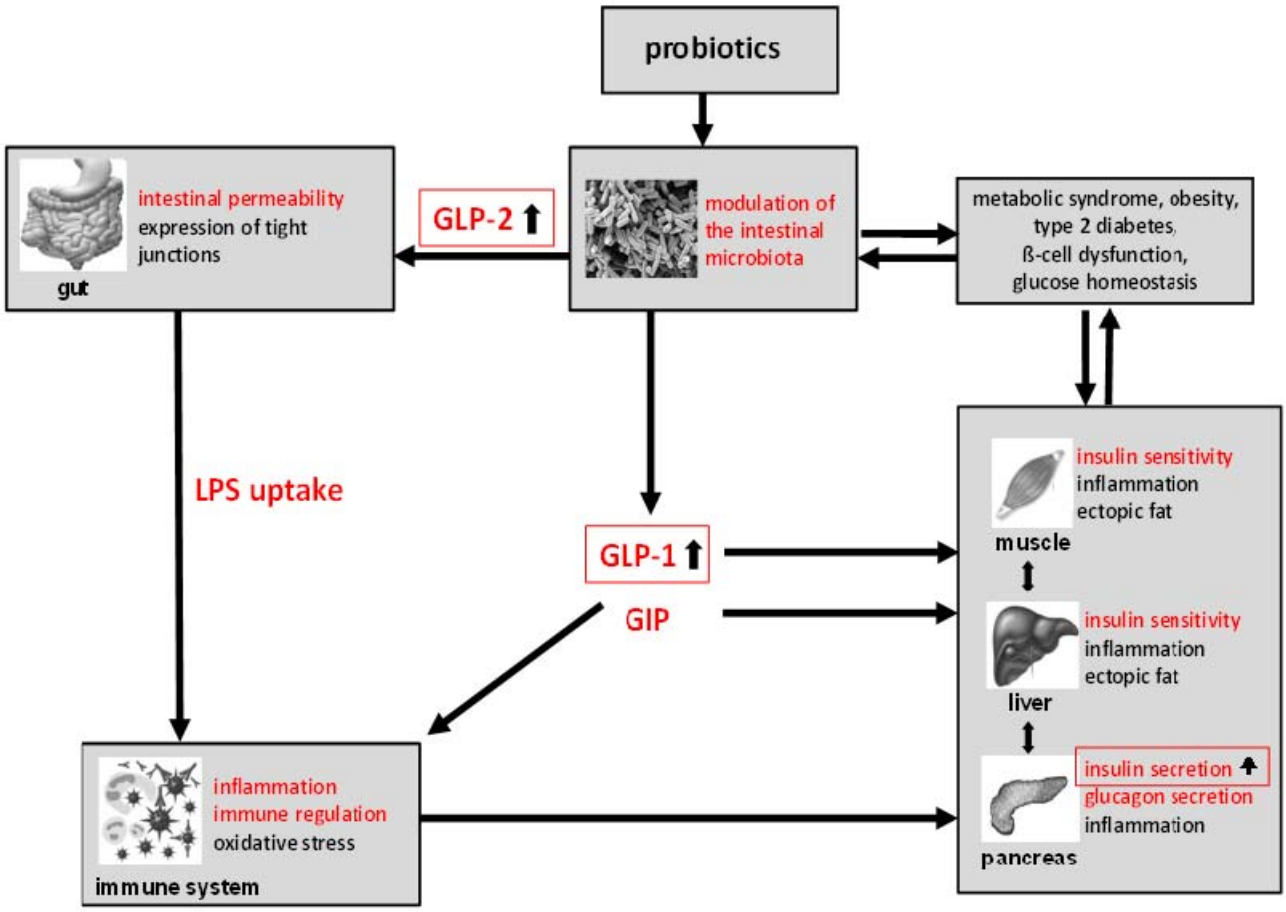

Fig. 26 Schematic overview of the probiotic effects
Specific questions which have been addressed are marked red, ↟ means improved effects. For further details see main text.

Good treatment adherence throughout the study and safety of the probiotic strain L. reuteri

Subjects who ingested the probiotic strain *L. reuteri* or placebo showed good compliance, which was confirmed by treatment adherence measured by capsule counting and detection of *L. reuteri* in fecal samples. Samples from persons ingesting *L. reuteri* were tested positive

for the presence of the bacteria, whereas placebo treated persons remained negative for *L. reuteri* in more than 90%, indicating very good treatment adherence and dietary restriction of other probiotic supplementation. Further, ingestion of *L. reuteri* and placebo was well tolerated, was safe in handling and dosage, and no side-effects were reported in either treatment arm.

Ingestion of the probiotic strain L. reuteri modulates plasma gut peptides

The incretin GLP-1 stimulate glucose-dependent the insulin secretion (Drucker, 2001b; Drucker, 2001a; Drucker, 2006). GLP-2 primarily targets the intestinal mucosa promoting growth and intestinal integrity (Drucker et al., 1996; Martin et al., 2005; Cani et al., 2009b). Both GLP-1 and GLP-2 are processed from proglucagon with equal efficiency and secreted by the intestinal L-cells simultaneously and in equimolar amounts (Drucker, 2002; Drucker, 2001b; Orskov et al., 1986; Holst JJ et al., 1997). Indeed, GLP-1 and GLP-2 concentrations measured in our study were correlated positively. Both gut hormones correlated intra-individually also with insulin and C-peptide concentrations during OGTT, but not during the isoglycemic test, as expected and suggestive for an efficient measurement of the incretin effect. The association of GLP-2 concentrations with those of insulin probably is indirect and can be explained by the co-secretion of GLP-2 and the incretin GLP-1. After treatment with *L. reuteri*, the mean increase of GLP-2 ΔAUC during OGTT was 43% compared to baseline, and this was parallel to the 76% increase seen for GLP-1. Probably only due to the limited number of participants, the ΔAUC GLP-1 increase was only significant compared to placebo but not within the intervention group. Therefore an involvement of both peptides, the intestinotrophic GLP-2 and the insulinotropic GLP-1, can be supposed. Our results complement the findings from an animal study, investigating the effects of a prebiotic treatment on the GLP-1 and GLP-2 concentrations (Cani et al., 2009b; Cani et al., 2005), and show that the probiotic strain *L. reuteri* increases the glucose-stimulated release of the incretin GLP-1 and the intestinotrophic GLP-2 in glucose tolerant human subjects.

In further animal models, testing prebiotics increased serum GLP-1 concentrations, lower fasting glucose, improved insulin sensitivity as indicated by HOMA-IR were reported. In this study pregnant Goto–Kakizaki rats were fed with dietary-resistant starch, suggesting that dietary-resistant starch with its known effects on the gut microbiota, improved maternal glycemic control (Shen et al., 2011). Similar results were obtained in a study with Wistar rats fed with prebiotic fiber. The treatment resulted in higher AUC GLP-1 levels during OGTT accompanied by significantly lower AUC glucose at the end of the study (Reimer et al., 2012). The only clinical trial, focusing on the effects of prebiotic treatment on incretins showed an association of prebiotic supplementation with increased GLP-1 and peptide YY concentration as well as with decreased postprandial plasma glucose responses after a

standardized meal in healthy human subjects (Cani et al., 2009a). However, no data exist regarding the effects of probiotic supplementation on incretin secretion. In our clinical trial we showed that administration of probiotic strain *L. reuteri* increased incretin, insulin and C-peptide responses in glucose-tolerant human subjects.

Administration of L. reuteri increases glucose-stimulated release of insulin and C-peptide

The insulinotropic gut-derived hormone GLP-1 shows decreased concentration and impaired function in type 2 diabetic subjects (Drucker, 2006; Wong and Brubaker, 2006; Vilsboll et al., 2001; Nauck et al., 1986). In our clinical trial with glucose-tolerant subjects, glucose stimulated GLP-1 concentrations were increased after administration of *L. reuteri*. Interestingly, the increase of GLP-1 levels occurred mainly in lean persons. However, the function of GLP-1, if defined as incretin effect (ΔAUC of GLP-1, insulin and C-peptide) was enhanced in the entire intervention group, suggesting that both lean and obese subjects may benefit from the intervention with *L. reuteri.*

Additionally, the effect of *L. reuteri* ingestion on the intestinal incretin response was accompanied by higher insulin and C-peptide responses during the OGTT, but not when the OGTT was mimicked by the isoglycemic intravenous glucose infusion. The mean increase of the ΔAUC insulin and C-peptide concentrations were elevated by 49% (46% for lean, 51% for obese individuals) and 55% (39% for lean, 74% for obese individuals), respectively, by the intervention. Thus, the incretin effect, as defined by changes of ΔAUC insulin and C-peptide, was improved by the intervention in our study cohort.

The gut hormones GLP-1 and GIP are proportionately released upon glucose or meal intake (Drucker, 2006; Holst JJ et al., 1997; Ferrannini et al., 1982) and they stimulate insulin secretion mediated by increasing blood glucose levels (Drucker, 2006; (Vilsboll et al., 2003a). However, the molecular mechanisms by which incretins stimulate insulin secretion have not been fully elucidated. Under physiological conditions, incretin-mediated stimulation of insulin secretion results from a generally enhanced beta-cell function (Muscelli et al., 2006). In patients with T2D, glucose- or meal-stimulated GLP-1 secretion is reduced compared to lean and obese non diabetic subjects (Vaag et al., 1996; Vilsboll et al., 2003b). In contrast, GLP-1 infusion stimulates insulin secretion in patients with T2D indistinguishable to control subjects (Kjems et al., 2003; Quddusi et al., 2003). This observation demonstrate, that the lack of GLP-1 secretion can counteracted by GLP-1 infusion, suggesting that the incretin mediated β-cell responsiveness to glucose is only partially affected, in patients with T2D.

In our clinical trial we showed that administration of *L. reuteri* leads to increased glucose-stimulated GLP-1 release, as well as increased insulin and C-peptide responses in lean and obese glucose-tolerant human subjects. Similarly, we observed alterations of indices of β-cell

function, indicating an improved β-cell function in *L. reuteri* treated persons, suggesting that increased insulin release is incretin mediated.

Ingestion of L. reuteri might accelerate intestinal motility

The majority of the intestinal K-cells secreting GIP are located in the proximal duodenum whereas the glucagon-like peptide secreting L-cells are located in the small and large intestine and GLP-1 is mainly synthesized and released from enteroendocrine L-cells in the distal ileum and colon (Martinez-Rodriguez and Gil, 2012; Dupre et al., 1973; Drucker, 2006; Drucker and Nauck, 2006). Since the concentrations of GIP remained unaffected, while the GLP-1 and GLP-2 secretion was increased, it may be concluded that the ingestion of *L. reuteri* might accelerate intestinal motility and improve mucosal functions. However, all recruited healthy subjects reported normal intestinal motility and stool frequency. This finding is in line with the results of a study investigating the effect of the consumption of a symbiotic product containing lactitol and *L. acidophilus* NCFM, twice daily over 2 weeks in a double-blind parallel trial with forty-seven healthy elderly subjects. This intervention only slightly increased stool frequency, improved intestinal motility and altered several markers suggestive for an improved mucosal integrity (Ouwehand et al., 2009).

Administration of L. reuteri had no impact on glucagon concentrations

The basic functions of glucagon is to counter-regulate the actions of insulin, and to maintaining physiological levels of blood glucose by inducing hepatic glucose production (Ali and Drucker, 2009). Glucagon may further reduce body weight by modifying food intake, glucose and lipid metabolism as well as energy expenditure. The impact of glucagon on all these physiological processes is mainly due to opposing the regulation of insulin (Lefebvre and Luyckx, 1969; Perea et al., 1995; Jensen et al., 1991; Tan et al., 2013).

In the development of type 2 diabetes fasting hyperglucagonemia is an early observation. The analysis of islet hormones in obese adolescent subjects revealed increased concentrations of fasting glucagon, particularly in subjects with insulin resistance or impaired glucose tolerance, while glucose or insulin appropriately suppressed glucagon secretion in these individuals (Weiss et al., 2011). In our study, investigating healthy, glucose tolerant subjects, the fasting concentrations of glucagon were higher in obese subjects compared to leans. However, there were no differences between lean and obese subjects under glucose stimulated conditions, and this was unaffected by the intervention with *L. reuteri*.

Furthermore, the incretin hormones GIP and GLP-1 seem to have divergent effects on glucagon secretion, while GIP seems to stimulate, GLP-1 seems to inhibit glucagon secretion (De Marinis et al., 2010; Pipeleers et al., 1985). We found a glucose-stimulated increase of GLP-1, while GIP and glucagon concentrations remained stable.

Effects of probiotic L. reuteri treatment on blood glucose concentrations

Ejtahed et al. reported from a human study that the consumption of a probiotic yoghurt containing *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 improved the levels of fasting blood glucose and HbA1c despite stable insulin concentrations and enzymatic antioxidant status in patients with type 2 diabetes, when compared to the control group which consumed conventional yoghurt (Ejtahed et al., 2012). Similarly, studies in pregnant women showed decreased blood glucose and less cases of gestational diabetes upon treatment with probiotics (Laitinen et al., 2009; Luoto et al., 2010). Laitinen et al. concluded that even in a normoglycemic population a dietary intervention with probiotics may improve blood glucose control (Laitinen et al., 2009). Our study in healthy lean and obese subjects with normal glucose tolerance did not show alterations of blood glucose or HbA1c, which may be due to the fact that we studied healthy subjects. However, our probiotic intervention improved the incretin and insulin serection even in this normoglycemic study population.

FFA levels seem unaffected by L. reuteri administration

Insulin sensitivity, insulin concentrations and glucose tolerance, are interrelated and each of these parameters may be modulated by changes in FFA concentrations (Newsholme et al., 2007; Reaven, 1988; Savage et al., 2007). Obese subjects, often exhibit elevated plasma FFA levels which may inhibit insulin-stimulated glucose uptake in the periphery. In non-diabetic humans FFA may stimulate insulin secretion and compensate for the FFA-mediated peripheral and hepatic insulin resistance (Boden, 2003; Boden, 1997). It has been postulated that G-protein-coupled receptors (GPRs) like GPR40, GPR120, whose endogenous ligands have been identified as long-chain free fatty acids (LCFA) (Hara et al., 2009; Hirasawa et al., 2005; Ichimura et al., 2012), mediate at least in part the observed increase in insulin secretion compensating for insulin resistance (Nagasumi et al., 2009; Kebede et al., 2008; Lan et al., 2008; Vettor et al., 2008). Consequently, if increased insulin secretion cannot be maintained, FFA effects cannot be counteracted and may lead to increased hepatic glucose production and reduced skeletal muscle glucose uptake as early signs of insulin resistance (Reaven, 1988).

In the present study, FFA concentrations were reduced by the glucose administration during OGTT, likely due to the increased insulin levels. There were no alterations of fasting and glucose-stimulated FFA concentrations due to intervention. Thus, *L. reuteri* modulates insulin secretion and gut peptide concentrations most likely without measurable FFA-mediated effects on whole body and hepatic insulin resistance.

It has been recognised that the GPRs contribute to the sensing of numerous metabolites such as fatty acids. Accordingly, it has been demonstrated that GPR40 and GRP120, who are mainly responsive to LCFA, are highly expressed by the K- and L-cells, and thereby could stimulate the GLP-1 and GIP secretion (Tanaka et al., 2008; Hirasawa et al., 2005). Thus, the digestion of TG to FFA is potentially involved in the induction of GLP-1 and GIP secretion (Parker et al., 2010). A study in mice by Edfalk et al. showed that GPR40 modulates FFA-stimulated insulin secretion from β-cells not only directly but also indirectly via regulation of incretin secretion (Edfalk et al., 2008).

In addition, related receptors like GPR41 and GPR43, responsive to short-chain fatty acids (SCFA), have been demonstrated to be expressed on L-cells as well (Tazoe et al., 2008) (Karaki et al., 2006) and may stimulate SCFA-triggered GLP-1 secretion at least *in vitro* (Tolhurst et al., 2012). This is of interest, since SCFA are products of bacterial fermentation of dietary fibre in the gut. Thus, it has been shown that SCFAs tend to increase the release of GLP-1 and PYY from the isolated perfused colon of rodents (Plaisancie et al., 1995; Longo et al., 1991).

In our clinical trial it has been demonstrated that ingestion of the probiotic strain *L. reuteri* increased GLP-1, insulin and C-peptide secretion, without affecting FFA concentrations in glucose-tolerant human subjects, this suggests that other metabolic mechanisms might be involved. However, in our project we did not measure GPRs expression and did not distinguish between LCFA and SCFA, as it was not the primary aim, but in further studies this possible mechanism needs to be investigated. When administration of probiotic strains is able to increase endogenous GLP-1 release from L-cells triggered by FFA, and this subsequently resulted in incretin-mediated insulin release, this may become an attractive alternative therapeutic strategy. Since the gut hormones not only affect gut physiology, but also glucose homeostasis, appetite and lipid metabolism. However, further studies are required to evaluate the therapeutic potential of this approach.

L. reuteri administration does not alter systemic and hepatic insulin sensitivity

Intrahepatocellular lipid accumulation correlates negatively with whole body and hepatic insulin sensitivity as demonstrated by impaired suppression of endogenous glucose production and decreased hepatic glycogen synthesis during hyperinsulinemic clamps (Krssak et al., 2004a; Bays et al., 2004). However, in our study there was no impact of *L. reuteri* administration on hepatic and peripheral insulin sensitivity as well as hepatocellular and myocellular lipid content. The only other study investigating effects of the consumption of *L. acidophilus* NCFM for four weeks on insulin sensitivity in a heterogeneous study population of 54 males with type 2 diabetes, or impaired or normal glucose tolerance, revealed preserved insulin sensitivity, when compared to the placebo group (Andreasen et

al., 2010a). in this study, there was considerable variability among study participants and it was not reported whether those with an increase of insulin sensitivity were diabetic or normoglycemic. Only in the latter case there would be a difference to our observations with glucose-tolerant subjects. Since, in our study glucose-tolerant subjects remained stable in systemic and hepatic insulin sensitivity.

Interestingly, Vrieze et al. recently investigated the effects of allogeneic fecal transplantation of lean donors on insulin sensitivity in 9 obese subjects with metabolic syndrome. They reported that allogeneic fecal transfer from lean donors to obese subjects improved peripheral insulin sensitivity, but had no effect on hepatic insulin sensitivity, body weight, and resting energy expenditure (Vrieze et al., 2012). Whereas this study reported an improvement of whole body insulin sensitivity, our results showed that whole body and hepatic insulin sensitivity as measured by hyperinsulinemic-euglycemic clamp with isotopic dilution remained unaltered upon intervention.

Impact of L. reuteri on fatty liver disease

As previous studies reported controversial results of probiotic treatment on fatty liver diseases. At least one study in humans suggested beneficial effects of probiotics on liver disease such as non-alcoholic fatty liver disease (NAFLD) and alcoholic liver cirrhosis (Loguercio et al., 2005), while in an open labelled pilot trial on 4 adult human subjects with NAFLD, all 4 subjects showed a significant increase in liver fat at the end of the intervention, with 3 of the 4 subjects exhibiting an increase of liver fat of more than 3% (Solga et al., 2008). Therefore, we carefully investigated the liver for potential side effects associated with ingestion of *L. reuteri.* In our study, the hepatocellular and myocellular lipid content of all participants remained unchanged, possibly due to the fact that we studied healthy subjects or maybe explained by a different treatment regimen as compared to the other studies.

In a study with rats, Wagnerberger et al. showed protective effects against the onset of fructose-induced NAFLD by *Lactobacillus casei* Shirota administration. In this study the protective effects of the probiotic strain seem to be mediated via a mechanism involving Toll-like receptor (TLR)-4 signalling in the liver (Wagnerberger et al., 2012). Since it is known that the stimulation of TLR-4 by the microbial component LPS induces a proinflammatory response (Lu et al., 2008; Kawai and Akira, 2006; Takeda and Akira, 2004), the results of Wagnerberger et al. suggest that the beneficial effects of the *Lactobacillus* strain are mediated either by a protection against the increased translocation of bacterial endotoxin or by attenuating the effects of endotoxin at the level of the liver (Wagnerberger et al., 2012). As in our study with healthy subjects the hepatocellular lipid content and the LPS concentrations were stable, we may conclude that the beneficial effects on the liver by *Lactobacillus* strains have their impact at the early stages of associated disease of NAFLD.

Intervention resulted in preserved concentrations of systemic inflammatory markers, oxidative stress and endotoxin

A cytokine panel of pro-inflammatory (IL-6, IL-8, IL-1β, IFN-γ, TNF-α, MCP-1, MIP-1β and regulatory (IL-10, IL1ra) cytokines and chemokines was assessed to investigate changes on the systemic inflammatory status following the intervention. As expected, lean subjects showed lower concentrations of hsCRP, IL-1ra, MCP-1, TNF-alpha compared to obese subjects, but surprisingly the detected cytokines and the TNF-α/IL-1ra ratio remained unaffected by the intervention. Four of the measured cytokines (IFN-γ, IL-6, IL-1β, and IL-10) were below the detection limit before and after the intervention, suggesting very low concentration of these in the systemic circulation, unaffected by intervention. This was against our expectations, since the cytokines IFN-γ, IL-6, IL-1β, and IL-10, are usually well detectable, even though in low concentrations in healthy subjects. While several other studies reported beneficial effects of probiotics on the inflammatory status (Rodes et al., 2013; Sun et al., 2013; Asemi et al., 2011; Boirivant and Strober, 2007; Calcinaro et al., 2005; Hakansson and Molin, 2011) as well as with regard to the ability to affect the systemic and mucosal immune system, intervention with probiotics seemed to be an accepted tool to modulate immune reactivity (McCarthy et al., 2003; Menard et al., 2004; Klaenhammer et al., 2012; Bischoff and Köchling, 2012; Jirillo et al., 2012; Lomax and Calder, 2009). The lack of alterations of systemic inflammation observed in our study may be due to the fact that we investigated healthy lean and obese subjects. *L. reuteri* intervention in subjects with type 2 diabetes might lead to different results, since patients with type 2 diabetes are associated with higher levels of systemic IL-1ra, IL-6 and TNF-α cytokines (Pham et al., 2011; Carstensen et al., 2010).

We also searched for changes of the inflammatory markers as consequence of the effects of the ingestion of *L. reuteri* on the gut peptides. An increase of GLP-2 secretion has been reported to enhance the expression of tight junctions in the intestinal wall, leading to decreased gut permeability and reduced leakage of endotoxin. These considerations lead to the assumption that circulating endotoxin levels are thought to be associated with gut permeability (Cani et al., 2009b; Pendyala et al., 2012; Erridge et al., 2007; Cani et al., 2007b; Cani et al., 2008a). It has been suggested that low-grade metabolic inflammation driven by endotoxin translocation due to increased intestinal permeability is the proposed mechanism by which the microbiota may contribute to systemic inflammation in obesity (Caesar et al., 2010; Delzenne and Cani, 2011; Hotamisligil, 2006; Li and Hotamisligil, 2010). Strains of *Lactobacillus spp.* may have the capability to improve the intestinal integrity in rodents, which may diminish the LPS-leakage from the gut to the circulation, and thereby reduce the systemic concentrations of inflammatory markers such as cytokines (Mao et al.,

1996; Luyer et al., 2005; Paturi et al., 2008). In our study however, LPS and systemic cytokine concentrations remained stable.

In an open-label randomized pilot trial, Leber et al. showed that the probiotic strain *L. casei* did not affect the gut permeability, but rather lead to a slight increase of the LPS-binding protein (LBP) and the hsCRP levels after three months of treatment (Leber et al., 2012). Interestingly, we detected a positive correlation of LPS with BMI before *L. reuteri* administration that was lost after intervention suggestive of potential changes of LPS translocation from the gut to the systemic circulation.

Effects of L. reuteri on intestinal permeability

Increased intestinal permeability is associated with increased translocation of endotoxins released from the gut microbiota into the systemic circulation. Further, in animal models a link between increased plasma endotoxin levels, and obesity and insulin resistance has been described (Cani et al., 2007a; Vajro et al., 2013; Scaldaferri et al., 2012; Teixeira et al., 2012).

However, the integrity of the intestinal barrier *in vivo* is difficult to measure and as already mentioned in the section 'material and method' there was no thoroughly evaluated and validated assay available to measure changes on a physiological level of intestinal permeability (A. Fasano, personal communication). Therefore, the conclusion that the detected tendency of reduced LPS translocation from the gut to systemic circulation is associated with changes in the intestinal permeability is highly speculative and needs to be addressed in further studies. These clinical trials should take the potential changes in the intestinal permeability by Lactobacillus (Leber et al., 2012) into account and focus on additional validated surrogate parameter along with systemic endotoxins levels.

Up to now, Ouwehand et al. reported that the ingestion of *Lactobacillus acidophilus* NCFM in combination with lactitol over 2 weeks altered the levels of Spermidine, a marker for an improved mucosal integrity and intestinal motility in healthy elderly subjects (Ouwehand et al., 2009). In contrast, in an open-label randomized pilot trial, Leber et al. showed that the probiotic strain *L. casei* does not affect the gut permeability in patients with metabolic disorders (Leber et al., 2012). Thus, these initial but conflicting evidence, whether the beneficial effects of probiotic microorganisms on intestinal barrier as reported in animal models (Wagnerberger et al., 2012; O'Hara and Shanahan, 2007; Iacono et al., 2011) and *in vitro* (Nissen et al., 2009) also occur in human subjects needs to be addressed in further clinical trials.

Effects of probiotic modulation of gut microbiota on adipokines

The two adipokines leptin and adiponectin are secreted by the white adipose tissue that has the capability to regulate insulin sensitivity (Xie et al., 2008) and participate in whole-body metabolism by affecting energy balance and glucose metabolism (Soodini, 2004; Lee et al., 2011; Morton and Schwartz, 2011). In obese mice, the modulation of gut microbiota improves leptin sensitivity, glucose homeostasis, and enteroendocrine cell activity (Everard et al., 2011). In addition, leptin has also been found to stimulate GLP-1 release from the human and rodent L-cells, and this effect is abolished in leptin-resistant diet-induced obese mice (Anini and Brubaker, 2003).

Further, leptin reduces intestinal mucosal adherence and translocation of bacteria in rodents, since in the absence of leptin, mice are obese and dramatically inflamed in the mesenteric adipose tissue (Amar et al., 2011). Lipopolysaccharide, derived from the gut bacteria, contributes to systemic inflammation and may contribute to impaired insulin sensitivity in humans. Whether leptin might be mechanistically involved in the LPS-mediated systemic inflammation derived from the gut microbiota and thereby contributes to impaired insulin sensitivity in human subjects needs to be investigated. In our clinical trial, leptin correlated positively with LPS in lean and obese subjects, and this association persisted after adjustment for BMI. Furthermore, leptin correlated inversely with intestinotrophic GLP-2, peripheral insulin sensitivity and positively with pro-inflammatory cytokines. Further, our study in healthy lean and obese subjects with normal glucose tolerance showed that ingestion of *L. reuteri* did not affect leptin and adiponectin, body mass, glucose tolerance or insulin sensitivity.

L. reuteri administration is associated with constant body weight and resting energy expenditure

Although our study failed to detect physiological consequences like improved insulin sensitivity, glucose tolerance and body weight, of an increased incretin, insulin and C-peptide response in the *L. reuteri* treated group, these cannot be excluded. With a treatment duration of 4 weeks, our study was not designed to test for long-term effects. In this context, it is noteworthy that two trials of long-term probiotic ingestion did observe decreased body weight and fat mass. Chang et al. found reduced body weight and BMI upon 8 week of intervention with a probiotic yogurt supplemented with a mixture of different bacterial strains in 101 healthy non-obese Korean subjects (Chang et al., 2011). Kadooka et al. reported a regulation of abdominal adiposity and body weight lowering effects by probiotics in 87 adults with tendencies of obesity (Kadooka et al., 2010).

The body weight of lean and obese subjects included in our study remained stable upon treatment with *L. reuteri* or placebo. Likewise, resting energy expenditure (REE), measured

by indirect calorimetry, remained stable. Furthermore, the REE and body weight before and after intervention showed an inverse intra-individual correlation with the AUCs of GLP-1 and GLP-2. These findings suggest that obese subjects have lower incretin concentrations compared to lean subjects during the glucose tolerance test. Differences with regard to *L. reuteri* treatment, however, have not been detected concerning to REE, neither in lean nor in obese subjects.

Strengths and limitations of the study

The limitations of our study were the small size of the cohort and the rather short intervention period and did not investigate patients with diagnosed diabetes. However, in contrast to other studies in the field, in which healthy and diseased subjects were analysed together, we investigated healthy, non-diabetic subjects and performed detailed metabolic characterization. We used strongly validated methods including the gold standard the hyperinsulinemic-euglycemic clamp test with isotopic dilution to assess tissue specific insulin sensitivity and substrate oxidation, the OGTT to determine the glucose tolerance, OGIS, and detailed calculations on insulin sensitivity and β-cell function.

One hypothesis is, that bacterial fermentation in the gut deliver SCFA, that may in turn modulate the release of gut hormones controlling insulin release and appetite (Tolhurst et al., 2012; Tarini and Wolever, 2010; Freeland et al., 2010). Short chain fatty acids play an important role in the development of type 2 diabetes, since it has been shown that butyrate-producing bacteria are decreased in patients with type 2 diabetes and subjects with metabolic disorders (Qin et al., 2012; Vrieze et al., 2012). As we did not investigate SCFA, this is one limitation of our study and it remains unclear, whether SCFA could be responsible for the changes of insulin secretion and incretin concentrations observed in our study. The measured FFA and triglycerides did not change upon intervention in our study cohort. However, the incretin effect was improved by the intervention which may reflect the known effects of gut microbiota and their fermentative end products, the SCFA.

The strength of our clinical trial is that we showed for the first time, that probiotic *L. reuteri* administration increased glucagon like peptides, insulin and C-peptide responses in glucose-tolerant human subjects suggestive of an incretin-mediated insulin release, however without improvement of glucose homeostasis and insulin sensitivity.

Conclusion

Subjects who ingested the probiotic strain *L. reuteri* or placebo showed good compliance, which was confirmed by treatment adherence and the detection of *L. reuteri* in the fecal samples of those subjects who received *L. reuteri*, while samples of subjects receiving placebo remained negative. The intake of *L. reuteri* was positively associated with *L. reuteri* containing feces and harmless changes in gut microbiota composition. Ingestion of *L. reuteri* showed no side effects, and was well tolerated.

As expected, lean and obese non-diabetic subjects differed with regard to body weight, whole body insulin sensitivity, hepatocellular lipid content and LPS concentrations before the intervention. The ingestion of *L. reuteri* over four weeks changed neither body mass and composition, nor ectopic fat content in particular liver fat content. Further, *L. reuteri* did not affect whole body and hepatic insulin sensitivity, systemic inflammation or oxidative stress but increased the release of C-peptide, insulin, and the gut hormones GLP-1 and GLP-2 in lean and obese glucose tolerant subjects.

These alterations of increased release of C-peptide, insulin, and the gut hormones GLP-1 and GLP-2 were detectable in non-diabetic humans after a rather short treatment period of only four weeks. Whether the moderate effects are continuable or enlarged after longer treatment periods remains to be addressed in long-term intervention studies.

Compared to findings from the literature, our study showed no effects of the ingestion of *L. reuteri* on peripheral and hepatic insulin sensitivity or related parameters such as body mass, ectopic fat content, and systemic inflammation or oxidative stress. But the increased insulin, C-peptide and glucagon-like peptides concentrations, suggest that the intervention has metabolic effects, even detectable in this small cohort of glucose tolerant subjects. Therefore, a more pronounced effect may be detectable in a more homogenous study group or a group with impaired glucose tolerance.

The observations implicate that there is a great potential of the administration of probiotics as a simple and efficient strategy to develop preventive and therapeutic approaches for type 2 diabetes. Thus, probiotic-mediated insulin secretion could serve as a novel therapeutic tool, which might be helpful in patients with type 2 diabetes or subjects with impaired glucose tolerance. However, further studies are required to establish, whether long-term treatment with *L. reuteri* or treatment of humans with overt diabetes mellitus will yield results that are of clinical benefit for the subjects with diabetes mellitus.

Outlook

The composition of the gut microbiota may affect various disorders like obesity, disturbed glucose homeostasis including insulin resistance and diabetes. The identification of different enterotypes is one way to look at and to classify people. Based on these findings, modulation of the gut microbiota can be considered as an option to prevent and treat diseases. To date the use of prebiotics and probiotics is one tool for educating the immune system and to prevent the development of metabolic disorders. These results are mainly established in animal models and many questions remain to be answered specially for human subjects.

The many reported heterogeneous findings may be attributed to factors such as different strains and dosages of probiotics, clinical characteristics of participants, duration of treatment period, sample size and study design. The present study demonstrates in a first step, that ingestion of *L. reuteri* modulates the secretion of insulin, C-peptide and the gut hormones GLP-1 and GLP-2 in humans, whereas no effects were observed on insulin sensitivity and parameters influencing insulin sensitivity such as body mass, ectopic fat content, and systemic inflammation or oxidative stress.

Since the modulation of gut microbiota and successional gut hormones not only affect gut physiology, but also glucose homeostasis, appetite and fat metabolism, the idea of administration of probiotic strains, who are able to increase incretin mediated insulin release would be an attractive area for drug development. The understanding on molecular mechanisms would facilitate this process. Therefore we have to think now more deeply how we can change the microbiome.

A personalized gut microbiota management may be an option for the prevention or even treatment of obesity, metabolic disorders and diabetes. However, how an efficient modulation of the gut microbiota of the host either by transplantation, administration of pre-, pro- or synbiotics or substitution with artificial optimized gut microbiota is optimally achieved needs to be investigated in further studies.

References

Abdul-Ghani,M.A. and Defronzo,R.A. (2009). Plasma glucose concentration and prediction of future risk of type 2 diabetes. Diabetes Care *32 Suppl 2*, S194-S198.

Abdul-Ghani,M.A., Jenkinson,C.P., Richardson,D.K., Tripathy,D., and Defronzo,R.A. (2006a). Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study. Diabetes *55*, 1430-1435.

Abdul-Ghani,M.A., Lyssenko,V., Tuomi,T., Defronzo,R.A., and Groop,L. (2010). The shape of plasma glucose concentration curve during OGTT predicts future risk of type 2 diabetes. Diabetes Metab Res. Rev. *26*, 280-286.

Abdul-Ghani,M.A., Tripathy,D., and Defronzo,R.A. (2006b). Contributions of beta-cell dysfunction and insulin resistance to the pathogenesis of impaired glucose tolerance and impaired fasting glucose. Diabetes Care *29*, 1130-1139.

Abrahamsson,T., Jakobsson,T., Sinkiewicz,G., Fredriksson,M., and Björkstén,B. (2005). Intestinal Microbiota in Infants Supplemented With the Probiotic Bacterium Lactobacillus Reuteri: Pn1-17. Journal of Pediatric Gastroenterology & Nutrition *40*, 692.

Abrahamsson,T.R., Jakobsson,T., Bottcher,M.F., Fredrikson,M., Jenmalm,M.C., Bjorksten,B., and Oldaeus,G. (2007). Probiotics in prevention of IgE-associated eczema: A double-blind, randomized, placebo-controlled trial. Journal of Allergy and Clinical Immunology *119*, 1174-1180.

ADA (2010). American Diabetes Association. Diagnosis and Classification of Diabetes mellitus. Diabetes Care *33*, 62-69.

Ahren,B. and Pacini,G. (1997). Impaired adaptation of first-phase insulin secretion in postmenopausal women with glucose intolerance. Am. J. Physiol *273*, E701-E707.

Ahren,B. and Pacini,G. (2004). Importance of quantifying insulin secretion in relation to insulin sensitivity to accurately assess beta cell function in clinical studies. Eur. J. Endocrinol. *150*, 97-104.

Al-Salami,H., Butt,G., Tucker,I., Skrbic,R., Golocorbin-Kon,S., and Mikov,M. (2008). Probiotic Pre-treatment Reduces Gliclazide Permeation (ex vivo) in Healthy Rats but Increases It in Diabetic Rats to the Level Seen in Untreated Healthy Rats. Arch. Drug Inf. *1*, 35-41.

Ali,S. and Drucker,D.J. (2009). Benefits and limitations of reducing glucagon action for the treatment of type 2 diabetes. Am. J. Physiol Endocrinol. Metab *296*, E415-E421.

Amar,J., Chabo,C., Waget,A., Klopp,P., Vachoux,C., Bermudez-Humaran,L.G., Smirnova,N., Berge,M., Sulpice,T., Lahtinen,S., Ouwehand,A., Langella,P., Rautonen,N., Sansonetti,P.J., and Burcelin,R. (2011). Intestinal mucosal adherence and translocation of commensal bacteria at the early onset of type 2 diabetes: molecular mechanisms and probiotic treatment. EMBO Mol. Med. *3*, 559-572.

Andersson,U., Branning,C., Ahrne,S., Molin,G., Alenfall,J., Onning,G., Nyman,M., and Holm,C. (2010). Probiotics lower plasma glucose in the high-fat fed C57BL/6J mouse. Benef. Microbes. *1*, 189-196.

Anderwald,C., Anderwald-Stadler,M., Promintzer,M., Prager,G., Mandl,M., Nowotny,P., Bischof,M.G., Wolzt,M., Ludvik,B., Kastenbauer,T., Pacini,G., Luger,A., and Krebs,M. (2007). The Clamp-Like Index: a novel and highly sensitive insulin sensitivity index to calculate hyperinsulinemic clamp glucose infusion rates from oral glucose tolerance tests in nondiabetic subjects. Diabetes Care *30*, 2374-2380.

Andreasen,A.S., Larsen,N., Pedersen-Skovsgaard,T., Berg,R.M.G., Moller,K., Svendsen,K.D., Jakobsen,M., and Pedersen,B.K. (2010a). Effects of Lactobacillus acidophilus NCFM on insulin sensitivity and the systemic inflammatory response in human subjects. British Journal of Nutrition *104*, 1831-1838.

Andreasen,A.S., Pedersen-Skovsgaard,T., Berg,R.M., Svendsen,K.D., Feldt-Rasmussen,B., Pedersen,B.K., and Moller,K. (2010b). Type 2 diabetes mellitus is associated with impaired cytokine response and adhesion molecule expression in human endotoxemia. Intensive Care Med. *36*, 1548-1555.

Anini,Y. and Brubaker,P.L. (2003). Role of leptin in the regulation of glucagon-like peptide-1 secretion. Diabetes *52*, 252-259.

Armato,J., Defronzo,R.A., Abdul-Ghani,M., and Ruby,R. (2012). Successful treatment of prediabetes in clinical practice: targeting insulin resistance and beta-cell dysfunction. Endocr. Pract. *18*, 342-350.

Asemi,Z., Jazayeri,S., Najafi,M., Samimi,M., Mofid,V., Shidfar,F., Foroushani,A.R., and Shahaboddin,M.E. (2011). Effects of daily consumption of probiotic yoghurt on inflammatory factors in pregnant women: a randomized controlled trial. Pak. J. Biol. Sci. *14*, 476-482.

Atkinson,B.J., Griesel,B.A., King,C.D., Josey,M.A., and Olson,A.L. (2013). Moderate GLUT4 overexpression improves insulin sensitivity and fasting triglyceridemia in high fat fed transgenic mice. Diabetes.

Axelsen,M., Smith,U., Eriksson,J.W., Taskinen,M.R., and Jansson,P.A. (1999). Postprandial hypertriglyceridemia and insulin resistance in normoglycemic first-degree relatives of patients with type 2 diabetes. Ann. Intern. Med. *131*, 27-31.

Badin,P.M., Vila,I.K., Louche,K., Mairal,A., Marques,M.A., Bourlier,V., Tavernier,G., Langin,D., and Moro,C. (2013). High-Fat Diet-Mediated Lipotoxicity and Insulin Resistance Is Related to Impaired Lipase Expression in Mouse Skeletal Muscle. Endocrinology.

Banks,W.A., Owen,J.B., and Erickson,M.A. (2012). Insulin in the brain: there and back again. Pharmacol. Ther. *136*, 82-93.

Bartholome,A.L., Albin,D.M., Baker,D.H., Holst,J.J., and Tappenden,K.A. (2004). Supplementation of total parenteral nutrition with butyrate acutely increases structural aspects of intestinal adaptation after an 80% jejunoileal resection in neonatal piglets. JPEN J. Parenter. Enteral Nutr. *28*, 210-222.

Bays,H., Mandarino,L., and Defronzo,R.A. (2004). Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. J. Clin. Endocrinol. Metab *89*, 463-478.

Benjamin,M.A., McKay,D.M., Yang,P.C., Cameron,H., and Perdue,M.H. (2000). Glucagon-like peptide-2 enhances intestinal epithelial barrier function of both transcellular and paracellular pathways in the mouse. Gut *47*, 112-119.

Bergman,R.N. (1989). Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes *38*, 1512-1527.

Bergman,R.N., Ader,M., Huecking,K., and Van,C.G. (2002). Accurate assessment of beta-cell function: the hyperbolic correction. Diabetes *51 Suppl 1*, S212-S220.

Bischoff,S.C. and Köchling,K. (2012). Pro- und Präbiotika. Aktuelle Ernährungsmedizin *37*, 287-304.

Bizhang,M., Ellerbrock,B., Preza,D., Raab,W., Singh,P., Beikler,T., Henrich,B., and Zimmer,S. (2011). Detection of nine microorganisms from the initial carious root lesions using a TaqMan-based real-time PCR. Oral Dis. *17*, 642-652.

Boden,G. (1997). Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes *46*, 3-10.

Boden,G. (2003). Effects of free fatty acids (FFA) on glucose metabolism: significance for insulin resistance and type 2 diabetes. Exp. Clin. Endocrinol. Diabetes *111*, 121-124.

Boirivant,M. and Strober,W. (2007). The mechanism of action of probiotics. Curr. Opin. Gastroenterol. *23*, 679-692.

Bonnet,F., Ducluzeau,P.H., Gastaldelli,A., Laville,M., Anderwald,C.H., Konrad,T., Mari,A., and Balkau,B. (2011). Liver enzymes are associated with hepatic insulin resistance, insulin secretion, and glucagon concentration in healthy men and women. Diabetes *60*, 1660-1667.

Bosi,E., Molteni,L., Radaelli,M.G., Folini,L., Fermo,I., Bazzigaluppi,E., Piemonti,L., Pastore,M.R., and Paroni,R. (2006). Increased intestinal permeability precedes clinical onset of type 1 diabetes. Diabetologia *49*, 2824-2827.

Bottcher,M.F., Abrahamsson,T.R., Fredriksson,M., Jakobsson,T., and Bjorksten,B. (2008). Low breast milk TGF-beta 2 is induced by Lactobacillus reuteri supplementation and associates with reduced risk of sensitization during infancy. Pediatric Allergy and Immunology *19*, 497-504.

Boushel,R., Gnaiger,E., Schjerling,P., Skovbro,M., Kraunsoe,R., and Dela,F. (2007). Patients with type 2 diabetes have normal mitochondrial function in skeletal muscle. Diabetologia *50*, 790-796.

Braden,B., Schröder,O., and Schneider,A. (2006). Bestimmung von Magenentleerung und Dünndarmtransit. In Funktionsdiagnostik in der Gastroenterologie, Springer Berlin Heidelberg), pp. 45-57.

Breneman,C.B. and Tucker,L. (2012). Dietary fibre consumption and insulin resistance - the role of body fat and physical activity. Br. J. Nutr. 1-9.

Brockman,D.A., Chen,X., and Gallaher,D.D. (2012). Hydroxypropyl methylcellulose, a viscous soluble fiber, reduces insulin resistance and decreases fatty liver in Zucker Diabetic Fatty rats. Nutr. Metab (Lond) *9*, 100.

Brubaker,P.L., Izzo,A., Hill,M., and Drucker,D.J. (1997). Intestinal function in mice with small bowel growth induced by glucagon-like peptide-2. Am. J. Physiol *272*, E1050-E1058.

Bruning,J.C., Winnay,J., Bonner-Weir,S., Taylor,S.I., Accili,D., and Kahn,C.R. (1997). Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. Cell *88*, 561-572.

Byun,R., Nadkarni,M.A., Chhour,K.L., Martin,F.E., Jacques,N.A., and Hunter,N. (2004). Quantitative analysis of diverse Lactobacillus species present in advanced dental caries. Journal of Clinical Microbiology *42*, 3128-3136.

Caesar,R., Fak,F., and Backhed,F. (2010). Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism. Journal of Internal Medicine *268*, 320-328.

Calanna,S., Christensen,M., Holst,J.J., Laferrere,B., Gluud,L.L., Vilsboll,T., and Knop,F.K. (2013). Secretion of glucagon-like peptide-1 in patients with type 2 diabetes mellitus: systematic review and meta-analyses of clinical studies. Diabetologia *56*, 965-972.

Calcinaro,F., Dionisi,S., Marinaro,M., Candeloro,P., Bonato,V., Marzotti,S., Corneli,R.B., Ferretti,E., Gulino,A., Grasso,F., De,S.C., Di,M.U., Falorni,A., Boirivant,M., and Dotta,F. (2005). Oral probiotic administration induces interleukin-10 production and prevents spontaneous autoimmune diabetes in the non-obese diabetic mouse. Diabetologia *48*, 1565-1575.

Cani,P.D., Amar,J., Iglesias,M.A., Poggi,M., Knauf,C., Bastelica,D., Neyrinck,A.M., Fava,F., Tuohy,K.M., Chabo,C., Waget,A., Delmee,E., Cousin,B., Sulpice,T., Chamontin,B., Ferrieres,J., Tanti,J.F., Gibson,G.R., Casteilla,L., Delzenne,N.M., Alessi,M.C., and Burcelin,R. (2007a). Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes *56*, 1761-1772.

Cani,P.D., Bibiloni,R., Knauf,C., Waget,A., Neyrinck,A.M., Delzenne,N.M., and Burcelin,R. (2008a). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes *57*, 1470-1481.

Cani,P.D. and Delzenne,N.M. (2009a). Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. Current Opinion in Pharmacology *9*, 737-743.

Cani,P.D. and Delzenne,N.M. (2009b). The Role of the Gut Microbiota in Energy Metabolism and Metabolic Disease. Current Pharmaceutical Design *15*, 1546-1558.

Cani,P.D. and Delzenne,N.M. (2009c). The role of the gut microbiota in energy metabolism and metabolic disease. Curr. Pharm. Des *15*, 1546-1558.

Cani,P.D., Delzenne,N.M., Amar,J., and Burcelin,R. (2008b). Role of gut microflora in the development of obesity and insulin resistance following high-fat diet feeding. Pathol. Biol. (Paris) *56*, 305-309.

Cani,P.D., Lecourt,E., Dewulf,E.M., Sohet,F.M., Pachikian,B.D., Naslain,D., De Backer,F., Neyrinck,A.M., and Delzenne,N.M. (2009a). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. American Journal of Clinical Nutrition *90*, 1236-1243.

Cani,P.D., Neyrinck,A.M., Fava,F., Knauf,C., Burcelin,R.G., Tuohy,K.M., Gibson,G.R., and Delzenne,N.M. (2007b). Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia *50*, 2374-2383.

Cani,P.D., Neyrinck,A.M., Maton,N., and Delzenne,N.M. (2005). Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like Peptide-1. Obes. Res. *13*, 1000-1007.

Cani,P.D., Osto,M., Geurts,L., and Everard,A. (2012). Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. Gut Microbes. *3*, 279-288.

Cani,P.D., Possemiers,S., Van de Wiele,T., Guiot,Y., Everard,A., Rottier,O., Geurts,L., Naslain,D., Neyrinck,A., Lambert,D.M., Muccioli,G.G., and Delzenne,N.M. (2009b). Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut *58*, 1091-1103.

Caradonna,L., Amati,L., Magrone,T., Pellegrino,N.M., Jirillo,E., and Caccavo,D. (2000). Enteric bacteria, lipopolysaccharides and related cytokines in inflammatory bowel disease: biological and clinical significance. J. Endotoxin. Res. *6*, 205-214.

Carstensen,M., Herder,C., Kivimaki,M., Jokela,M., Roden,M., Shipley,M.J., Witte,D.R., Brunner,E.J., and Tabak,A.G. (2010). Accelerated increase in serum interleukin-1 receptor antagonist starts 6 years before diagnosis of type 2 diabetes: Whitehall II prospective cohort study. Diabetes *59*, 1222-1227.

Chan,J.C., Malik,V., Jia,W., Kadowaki,T., Yajnik,C.S., Yoon,K.H., and Hu,F.B. (2009). Diabetes in Asia: epidemiology, risk factors, and pathophysiology. JAMA *301*, 2129-2140.

Chang,B.J., Park,S.U., Jang,Y.S., Ko,S.H., Joo,N.M., Kim,S.I., Kim,C.H., and Chang,D.K. (2011). Effect of functional yogurt NY-YP901 in improving the trait of metabolic syndrome. European Journal of Clinical Nutrition *65*, 1250-1255.

Cheeseman,C.I. (1997). Upregulation of SGLT-1 transport activity in rat jejunum induced by GLP-2 infusion in vivo. Am. J. Physiol *273*, R1965-R1971.

Chen,H., Sullivan,G., Yue,L.Q., Katz,A., and Quon,M.J. (2003). QUICKI is a useful index of insulin sensitivity in subjects with hypertension. Am. J. Physiol Endocrinol. Metab *284*, E804-E812.

Chen,J.J., Wang,R., Li,X.F., and Wang,R.L. (2011). Bifidobacterium longum supplementation improved high-fat-fed-induced metabolic syndrome and promoted intestinal Reg I gene expression. Exp. Biol. Med. (Maywood.) *236*, 823-831.

Cloetens,L., Ulmius,M., Johansson-Persson,A., Akesson,B., and Onning,G. (2012). Role of dietary beta-glucans in the prevention of the metabolic syndrome. Nutr. Rev. *70*, 444-458.

Dastych,M., Dastych,M., Jr., Novotna,H., and Cihalova,J. (2008). Lactulose/mannitol test and specificity, sensitivity, and area under curve of intestinal permeability parameters in patients with liver cirrhosis and Crohn's disease. Dig. Dis. Sci. *53*, 2789-2792.

De Marinis,Y.Z., Salehi,A., Ward,C.E., Zhang,Q., Abdulkader,F., Bengtsson,M., Braha,O., Braun,M., Ramracheya,R., Amisten,S., Habib,A.M., Moritoh,Y., Zhang,E., Reimann,F., Rosengren,A.H., Shibasaki,T., Gribble,F., Renstrom,E., Seino,S., Eliasson,L., and Rorsman,P. (2010). GLP-1 inhibits and adrenaline stimulates glucagon release by differential modulation of N- and L-type Ca2+ channel-dependent exocytosis. Cell Metab *11*, 543-553.

Deacon,C.F. and Holst,J.J. (2009). Immunoassays for the incretin hormones GIP and GLP-1. Best. Pract. Res. Clin. Endocrinol. Metab *23*, 425-432.

Defronzo,R.A. (2004). Pathogenesis of type 2 diabetes mellitus. Med. Clin. North Am. *88*, 787-835, ix.

Defronzo,R.A. (2009). Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes *58*, 773-795.

Defronzo,R.A., Tobin,J.D., and Andres,R. (1979). Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am. J. Physiol *237*, E214-E223.

Defronzo,R.A. and Tripathy,D. (2009). Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care *32 Suppl 2*, S157-S163.

Dela,F., Larsen,J.J., Mikines,K.J., Ploug,T., Petersen,L.N., and Galbo,H. (1995). Insulin-stimulated muscle glucose clearance in patients with NIDDM. Effects of one-legged physical training. Diabetes *44*, 1010-1020.

Delzenne,N.M. and Cani,P.D. (2011). Interaction Between Obesity and the Gut Microbiota: Relevance in Nutrition. Annual Review of Nutrition, Vol 31 *31*, 15-31.

Drucker,D.J. (2001a). Development of glucagon-like peptide-1-based pharmaceuticals as therapeutic agents for the treatment of diabetes. Curr. Pharm. Des *7*, 1399-1412.

Drucker,D.J. (2001b). Minireview: the glucagon-like peptides. Endocrinology *142*, 521-527.

Drucker,D.J. (2002). Biological actions and therapeutic potential of the glucagon-like peptides. Gastroenterology *122*, 531-544.

Drucker,D.J. (2006). The biology of incretin hormones. Cell Metab *3*, 153-165.

Drucker,D.J., Ehrlich,P., Asa,S.L., and Brubaker,P.L. (1996). Induction of intestinal epithelial proliferation by glucagon-like peptide 2. Proceedings of the National Academy of Sciences of the United States of America *93*, 7911-7916.

Drucker,D.J. and Nauck,M.A. (2006). The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. Lancet *368*, 1696-1705.

Dupre,J., Ross,S.A., Watson,D., and Brown,J.C. (1973). Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J. Clin. Endocrinol. Metab *37*, 826-828.

Edfalk,S., Steneberg,P., and Edlund,H. (2008). Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. Diabetes *57*, 2280-2287.

Ejtahed,H.S., Mohtadi-Nia,J., Homayouni-Rad,A., Niafar,M., Asghari-Jafarabadi,M., and Mofid,V. (2012). Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition *28*, 539-543.

ELRICK,H., STIMMLER,L., HLAD,C.J., Jr., and ARAI,Y. (1964). PLASMA INSULIN RESPONSE TO ORAL AND INTRAVENOUS GLUCOSE ADMINISTRATION. J. Clin. Endocrinol. Metab *24*, 1076-1082.

Erridge,C., Attina,T., Spickett,C.M., and Webb,D.J. (2007). A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am. J. Clin. Nutr. *86*, 1286-1292.

Everard,A., Lazarevic,V., Derrien,M., Girard,M., Muccioli,G.G., Neyrinck,A.M., Possemiers,S., Van,H.A., Francois,P., de Vos,W.M., Delzenne,N.M., Schrenzel,J., and Cani,P.D. (2011). Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes *60*, 2775-2786.

Faerch,K., Vaag,A., Holst,J.J., Glumer,C., Pedersen,O., and Borch-Johnsen,K. (2008). Impaired fasting glycaemia vs impaired glucose tolerance: similar impairment of pancreatic alpha and beta cell function but differential roles of incretin hormones and insulin action. Diabetologia *51*, 853-861.

Fantuzzi,G. (2005). Adipose tissue, adipokines, and inflammation. J. Allergy Clin. Immunol. *115*, 911-919.

Ferrannini,E. (2009). Insulin resistance versus beta-cell dysfunction in the pathogenesis of type 2 diabetes. Curr. Diab. Rep. *9*, 188-189.

Ferrannini,E., Barrett,E., Bevilacqua,S., Dupre,J., and Defronzo,R.A. (1982). Sodium elevates the plasma glucose response to glucose ingestion in man. J. Clin. Endocrinol. Metab *54*, 455-458.

Ferrannini,E., Gastaldelli,A., Miyazaki,Y., Matsuda,M., Mari,A., and Defronzo,R.A. (2005). beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: a new analysis. J. Clin. Endocrinol. Metab *90*, 493-500.

Festa,A., Williams,K., D'Agostino,R., Jr., Wagenknecht,L.E., and Haffner,S.M. (2006). The natural course of beta-cell function in nondiabetic and diabetic individuals: the Insulin Resistance Atherosclerosis Study. Diabetes *55*, 1114-1120.

Frayn,K.N. (1983). Calculation of Substrate Oxidation Rates Invivo from Gaseous Exchange. Journal of Applied Physiology *55*, 628-634.

Freeland,K.R., Wilson,C., and Wolever,T.M.S. (2010). Adaptation of colonic fermentation and glucagon-like peptide-1 secretion with increased wheat fibre intake for 1 year in hyperinsulinaemic human subjects. British Journal of Nutrition *103*, 82-90.

Galisteo,M., Duarte,J., and Zarzuelo,A. (2008). Effects of dietary fibers on disturbances clustered in the metabolic syndrome. J. Nutr. Biochem. *19*, 71-84.

Geloneze,B., Vasques,A.C., Stabe,C.F., Pareja,J.C., Rosado,L.E., Queiroz,E.C., and Tambascia,M.A. (2009). HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS). Arq Bras. Endocrinol. Metabol. *53*, 281-287.

Genta,S., Cabrera,W., Habib,N., Pons,J., Carillo,I.M., Grau,A., and Sanchez,S. (2009). Yacon syrup: Beneficial effects on obesity and insulin resistance in humans. Clinical Nutrition *28*, 182-187.

Gerritsen,J., Smidt,H., Rijkers,G.T., and de Vos,W.M. (2011). Intestinal microbiota in human health and disease: the impact of probiotics. Genes and Nutrition *6*, 209-240.

Groop,L., Forsblom,C., Lehtovirta,M., Tuomi,T., Karanko,S., Nissen,M., Ehrnstrom,B.O., Forsen,B., Isomaa,B., Snickars,B., and Taskinen,M.R. (1996). Metabolic consequences of a family history of NIDDM (the Botnia study): evidence for sex-specific parental effects. Diabetes *45*, 1585-1593.

Guan,X., Shi,X., Li,X., Chang,B., Wang,Y., Li,D., and Chan,L. (2012). GLP-2 receptor in POMC neurons suppresses feeding behavior and gastric motility. Am. J. Physiol Endocrinol. Metab *303*, E853-E864.

Guilherme,A., Virbasius,J.V., Puri,V., and Czech,M.P. (2008). Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat. Rev. Mol. Cell Biol. *9*, 367-377.

Haarman,M. and Knol,J. (2006). Quantitative real-time PCR analysis of fecal Lactobacillus species in infants receiving a prebiotic infant formula. Applied and Environmental Microbiology *72*, 2359-2365.

Habich,C., Kempe,K., van der Zee,R., Rumenapf,R., Akiyama,H., Kolb,H., and Burkart,V. (2005). Heat shock protein 60: Specific binding of lipopolysaccharide. Journal of Immunology *174*, 1298-1305.

Hakansson,A. and Molin,G. (2011). Gut microbiota and inflammation. Nutrients. *3*, 637-682.

Hamilton,G., Yokoo,T., Bydder,M., Cruite,I., Schroeder,M.E., Sirlin,C.B., and Middleton,M.S. (2011). In vivo characterization of the liver fat (1)H MR spectrum. NMR Biomed. *24*, 784-790.

Hara,T., Hirasawa,A., Sun,Q., Sadakane,K., Itsubo,C., Iga,T., Adachi,T., Koshimizu,T.A., Hashimoto,T., Asakawa,Y., and Tsujimoto,G. (2009). Novel selective ligands for free fatty acid receptors GPR120 and GPR40. Naunyn Schmiedebergs Arch. Pharmacol. *380*, 247-255.

Harford,K.A., Reynolds,C.M., McGillicuddy,F.C., and Roche,H.M. (2011). Fats, inflammation and insulin resistance: insights to the role of macrophage and T-cell accumulation in adipose tissue. Proc. Nutr. Soc. *70*, 408-417.

Hartmann,B., Johnsen,A.H., Orskov,C., Adelhorst,K., Thim,L., and Holst,J.J. (2000). Structure, measurement, and secretion of human glucagon-like peptide-2. Peptides *21*, 73-80.

Heini,A.F., Lara-Castro,C., Schneider,H., Kirk,K.A., Considine,R.V., and Weinsier,R.L. (1998). Effect of hydrolyzed guar fiber on fasting and postprandial satiety and satiety hormones: a double-blind, placebo-controlled trial during controlled weight loss. Int. J. Obes. Relat Metab Disord. *22*, 906-909.

Hemminki,K., Li,X., Sundquist,K., and Sundquist,J. (2010). Familial risks for type 2 diabetes in Sweden. Diabetes Care *33*, 293-297.

Herder,C., Brunner,E.J., Rathmann,W., Strassburger,K., Tabak,A.G., Schloot,N.C., and Witte,D.R. (2009a). Elevated Levels of the Anti-Inflammatory Interleukin-1 Receptor Antagonist Precede the Onset of Type 2 Diabetes. Diabetes Care *32*, 421-423.

Herder,C., Haastert,B., Muller-Scholze,S., Koenig,W., Thorand,B., Holle,R., Wichmann,H.E., Scherbaum,W.A., Martin,S., and Kolb,H. (2005a). Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes - Results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). Diabetes *54*, S11-S17.

Herder,C., Illig,T., Rathmann,W., Martin,S., Haastert,B., Muller-Scholze,S., Holle,R., Thorand,B., Koenig,W., Wichmann,H.E., and Kolb,H. (2005b). Inflammation and type 2 diabetes: Results from KORA Augsburg. Gesundheitswesen *67*, S115-S121.

Herder,C. and Roden,M. (2011). Genetics of type 2 diabetes: pathophysiologic and clinical relevance. Eur. J. Clin. Invest *41*, 679-692.

Herder,C., Schmitz-Beuting,C., Rathmann,W., Haastert,B., Schmitz-Beuting,J., Schafer,M., Scherbaum,W.A., Schneitler,H., and Martin,S. (2007a). Prevalence of impaired glucose regulation in German school-leaving students. International Journal of Obesity *31*, 1086-1088.

Herder,C., Schneitler,S., Rathmann,W., Haastert,B., Schneitler,H., Winkler,H., Bredahl,R., Hahnloser,E., and Martin,S. (2007b). Low-grade inflammation, obesity, and insulin resistance in adolescents. Journal of Clinical Endocrinology & Metabolism *92*, 4569-4574.

Herder,C., Zierer,A., Koenig,W., Roden,M., Meisinger,C., and Thorand,B. (2009b). Transforming Growth Factor-beta 1 and Incident Type 2 Diabetes Results from the MONICA/KORA case-cohort study, 1984-2002. Diabetes Care *32*, 1921-1923.

Hirasawa,A., Tsumaya,K., Awaji,T., Katsuma,S., Adachi,T., Yamada,M., Sugimoto,Y., Miyazaki,S., and Tsujimoto,G. (2005). Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat. Med. *11*, 90-94.

Hlivak,P., Odraska,J., Ferencik,M., Ebringer,L., Jahnova,E., and Mikes,Z. (2005). One-year application of probiotic strain Enterococcus faecium M-74 decreases serum cholesterol levels. Bratisl. Lek. Listy *106*, 67-72.

Hoffmann,M., Rath,E., Holzlwimmer,G., Quintanilla-Martinez,L., Loach,D., Tannock,G., and Haller,D. (2008). Lactobacillus reuteri 100-23 transiently activates intestinal epithelial cells of mice that have a complex microbiota during early stages of colonization. Journal of Nutrition *138*, 1684-1691.

Holst JJ, Ørskov C, Hartmann B, and Deacon CF. (1997). Posttranslational processing of proglucagon and postsecretory fate of proglucagon products. In The insulinotropic gut hormone glucagon-like peptide-1., Fehmann HC and Göke B , eds. (Basel: Karger), pp. 24-48.

Hotamisligil,G.S. (2006). Inflammation and metabolic disorders. Nature *444*, 860-867.

Hrebicek,J., Janout,V., Malincikova,J., Horakova,D., and Cizek,L. (2002). Detection of insulin resistance by simple quantitative insulin sensitivity check index QUICKI for epidemiological assessment and prevention. J. Clin. Endocrinol. Metab *87*, 144-147.

Hwang,J.H., Stein,D.T., Barzilai,N., Cui,M.H., Tonelli,J., Kishore,P., and Hawkins,M. (2007). Increased intrahepatic triglyceride is associated with peripheral insulin resistance: in vivo MR imaging and spectroscopy studies. American Journal of Physiology-Endocrinology and Metabolism *293*, E1663-E1669.

Iacono,A., Raso,G.M., Canani,R.B., Calignano,A., and Meli,R. (2011). Probiotics as an emerging therapeutic strategy to treat NAFLD: focus on molecular and biochemical mechanisms. J. Nutr. Biochem. *22*, 699-711.

Ichimura,A., Hirasawa,A., Poulain-Godefroy,O., Bonnefond,A., Hara,T., Yengo,L., Kimura,I., Leloire,A., Liu,N., Iida,K., Choquet,H., Besnard,P., Lecoeur,C., Vivequin,S., Ayukawa,K., Takeuchi,M., Ozawa,K., Tauber,M., Maffeis,C., Morandi,A., Buzzetti,R., Elliott,P., Pouta,A., Jarvelin,M.R., Korner,A., Kiess,W., Pigeyre,M., Caiazzo,R., Van,H.W., Van,G.L., Horber,F., Balkau,B., Levy-Marchal,C., Rouskas,K., Kouvatsi,A., Hebebrand,J., Hinney,A., Scherag,A., Pattou,F., Meyre,D., Koshimizu,T.A., Wolowczuk,I., Tsujimoto,G., and Froguel,P. (2012). Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. Nature *483*, 350-354.

IDF. International Diabetes Federation's 5th edition of the Diabetes Atlas 2011. 2011.
Ref Type: Online Source

Indrio,F., Riezzo,G., Raimondi,F., Bisceglia,M., Cavallo,L., and Francavilla,R. (2008). The effects of probiotics on feeding tolerance, bowel habits, and gastrointestinal motility in preterm newborns. Journal of Pediatrics *152*, 801-806.

Jacobsen,C.N., Rosenfeldt,N., V, Hayford,A.E., Moller,P.L., Michaelsen,K.F., Paerregaard,A., Sandstrom,B., Tvede,M., and Jakobsen,M. (1999). Screening of probiotic activities of forty-seven strains of Lactobacillus spp. by in vitro techniques and evaluation of the colonization ability of five selected strains in humans. Appl. Environ. Microbiol. *65*, 4949-4956.

Jensen,M.D., Heiling,V.J., and Miles,J.M. (1991). Effects of glucagon on free fatty acid metabolism in humans. J. Clin. Endocrinol. Metab *72*, 308-315.

Jeppesen,P.B., Sanguinetti,E.L., Buchman,A., Howard,L., Scolapio,J.S., Ziegler,T.R., Gregory,J., Tappenden,K.A., Holst,J., and Mortensen,P.B. (2005). Teduglutide (ALX-0600), a dipeptidyl peptidase IV resistant glucagon-like peptide 2 analogue, improves intestinal function in short bowel syndrome patients. Gut *54*, 1224-1231.

Jirillo,E., Jirillo,F., and Magrone,T. (2012). Healthy effects exerted by prebiotics, probiotics, and symbiotics with special reference to their impact on the immune system. Int. J. Vitam. Nutr. Res. *82*, 200-208.

Kadooka,Y., Sato,M., Imaizumi,K., Ogawa,A., Ikuyama,K., Akai,Y., Okano,M., Kagoshima,M., and Tsuchida,T. (2010). Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur. J. Clin. Nutr. *64*, 636-643.

Kahn,S.E., Beard,J.C., Schwartz,M.W., Ward,W.K., Ding,H.L., Bergman,R.N., Taborsky,G.J., Jr., and Porte,D., Jr. (1989). Increased beta-cell secretory capacity as mechanism for islet adaptation to nicotinic acid-induced insulin resistance. Diabetes *38*, 562-568.

Kahn,S.E., Prigeon,R.L., McCulloch,D.K., Boyko,E.J., Bergman,R.N., Schwartz,M.W., Neifing,J.L., Ward,W.K., Beard,J.C., Palmer,J.P., and . (1993). Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. Diabetes *42*, 1663-1672.

Kaprio,J., Tuomilehto,J., Koskenvuo,M., Romanov,K., Reunanen,A., Eriksson,J., Stengard,J., and Kesaniemi,Y.A. (1992). Concordance for type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetes mellitus in a population-based cohort of twins in Finland. Diabetologia *35*, 1060-1067.

Karaki,S., Mitsui,R., Hayashi,H., Kato,I., Sugiya,H., Iwanaga,T., Furness,J.B., and Kuwahara,A. (2006). Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. Cell Tissue Res. *324*, 353-360.

Kashyap,S.R. and Defronzo,R.A. (2007). The insulin resistance syndrome: physiological considerations. Diab. Vasc. Dis. Res. *4*, 13-19.

Kato,Y., Yu,D., and Schwartz,M.Z. (1999). Glucagonlike peptide-2 enhances small intestinal absorptive function and mucosal mass in vivo. J. Pediatr. Surg. *34*, 18-20.

Katz,A., Nambi,S.S., Mather,K., Baron,A.D., Follmann,D.A., Sullivan,G., and Quon,M.J. (2000). Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J. Clin. Endocrinol. Metab *85*, 2402-2410.

Kawai,T. and Akira,S. (2006). TLR signaling. Cell Death. Differ. *13*, 816-825.

Kebede,M., Alquier,T., Latour,M.G., Semache,M., Tremblay,C., and Poitout,V. (2008). The fatty acid receptor GPR40 plays a role in insulin secretion in vivo after high-fat feeding. Diabetes *57*, 2432-2437.

Kelley,D.E., He,J., Menshikova,E.V., and Ritov,V.B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes *51*, 2944-2950.

Kempf,K., Rose,B., Herder,C., Haastert,B., Fusbahn-Laufenburg,A., Reifferscheid,A., Scherbaum,W.A., Kolb,H., and Martin,S. (2007). The metabolic syndrome sensitizes leukocytes for glucose-induced immune gene expression. Journal of Molecular Medicine-Jmm *85*, 389-396.

Kendall,D.M., Cuddihy,R.M., and Bergenstal,R.M. (2009). Clinical application of incretin-based therapy: therapeutic potential, patient selection and clinical use. Eur. J. Intern. Med. *20 Suppl 2*, S329-S339.

King,D.S., Dalsky,G.P., Staten,M.A., Clutter,W.E., Van Houten,D.R., and Holloszy,J.O. (1987). Insulin action and secretion in endurance-trained and untrained humans. J. Appl. Physiol *63*, 2247-2252.

Kjems,L.L., Holst,J.J., Volund,A., and Madsbad,S. (2003). The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects. Diabetes *52*, 380-386.

Klaenhammer,T.R., Kleerebezem,M., Kopp,M.V., and Rescigno,M. (2012). The impact of probiotics and prebiotics on the immune system. Nat. Rev. Immunol. *12*, 728-734.

Kolb,H. and Mandrup-Poulsen,T. (2010). The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. Diabetologia *53*, 10-20.

Korhonen,H. and Pihlanto,A. (2006). Bioactive peptides: Production and functionality. International Dairy Journal *16*, 945-960.

Krebs,M., Krssak,M., Nowotny,P., Weghuber,D., Gruber,S., Mlynarik,V., Bischof,M., Stingl,H., Furnsinn,C., Waldhausl,W., and Roden,M. (2001). Free fatty acids inhibit the glucose-stimulated increase of intramuscular glucose-6-phosphate concentration in humans. Journal of Clinical Endocrinology & Metabolism *86*, 2153-2160.

Krssak,M., Brehm,A., Bernroider,E., Anderwald,C., Nowotny,P., Dalla,M.C., Cobelli,C., Cline,G.W., Shulman,G.I., Waldhausl,W., and Roden,M. (2004a). Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. Diabetes *53*, 3048-3056.

Krssak,M., Falk,P.K., Dresner,A., DiPietro,L., Vogel,S.M., Rothman,D.L., Roden,M., and Shulman,G.I. (1999). Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. Diabetologia *42*, 113-116.

Krssak,M., Hofer,H., Wrba,F., Meyerspeer,M., Brehm,A., Lohninger,A., Steindl-Munda,P., Moser,E., Ferenci,P., and Roden,M. (2010). Non-invasive assessment of hepatic fat accumulation in chronic hepatitis C by 1H magnetic resonance spectroscopy. Eur. J. Radiol. *74*, e60-e66.

Krssak,M., Mlynarik,V., Meyerspeer,M., Moser,E., and Roden,M. (2004b). 1H NMR relaxation times of skeletal muscle metabolites at 3 T. MAGMA. *16*, 155-159.

Laitinen,K., Poussa,T., and Isolauri,E. (2009). Probiotics and dietary counselling contribute to glucose regulation during and after pregnancy: a randomised controlled trial. British Journal of Nutrition *101*, 1679-1687.

Lan,H., Hoos,L.M., Liu,L., Tetzloff,G., Hu,W., Abbondanzo,S.J., Vassileva,G., Gustafson,E.L., Hedrick,J.A., and Davis,H.R. (2008). Lack of FFAR1/GPR40 does not protect mice from high-fat diet-induced metabolic disease. Diabetes *57*, 2999-3006.

Larsen,N., Vogensen,F.K., van den Berg,F.W.J., Nielsen,D.S., Andreasen,A.S., Pedersen,B.K., Abu Al-Soud,W., Sorensen,S.J., Hansen,L.H., and Jakobsen,M. (2010). Gut Microbiota in Human Adults with Type 2 Diabetes Differs from Non-Diabetic Adults. Plos One *5*.

Lauro,D., Kido,Y., Castle,A.L., Zarnowski,M.J., Hayashi,H., Ebina,Y., and Accili,D. (1998). Impaired glucose tolerance in mice with a targeted impairment of insulin action in muscle and adipose tissue. Nat. Genet. *20*, 294-298.

Leber,B., Tripolt,N.J., Blattl,D., Eder,M., Wascher,T.C., Pieber,T.R., Stauber,R., Sourij,H., Oettl,K., and Stadlbauer,V. (2012). The influence of probiotic supplementation on gut permeability in patients with metabolic syndrome: an open label, randomized pilot study. Eur. J. Clin. Nutr. *66*, 1110-1115.

Lee,Y.H., Magkos,F., Mantzoros,C.S., and Kang,E.S. (2011). Effects of leptin and adiponectin on pancreatic beta-cell function. Metabolism *60*, 1664-1672.

Lefebvre,P.J. and Luyckx,A.S. (1969). Effect of insulin on glucagon enhanced lipolysis in vitro. Diabetologia *5*, 195-197.

Lettner,A. and Roden,M. (2008). Ectopic fat and insulin resistance. Curr. Diab. Rep. *8*, 185-191.

Levy,J.C., Matthews,D.R., and Hermans,M.P. (1998). Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care *21*, 2191-2192.

Ley,R.E., Backhed,F., Turnbaugh,P., Lozupone,C.A., Knight,R.D., and Gordon,J.I. (2005). Obesity alters gut microbial ecology. Proceedings of the National Academy of Sciences of the United States of America *102*, 11070-11075.

Li,P. and Hotamisligil,G.S. (2010). METABOLISM Host and microbes in a pickle. Nature *464*, 1287-1288.

Lillioja,S., Mott,D.M., Howard,B.V., Bennett,P.H., Yki-Jarvinen,H., Freymond,D., Nyomba,B.L., Zurlo,F., Swinburn,B., and Bogardus,C. (1988). Impaired glucose tolerance as a disorder of insulin action. Longitudinal and cross-sectional studies in Pima Indians. N. Engl. J. Med. *318*, 1217-1225.

Liu,Y.Y., Fatheree,N.Y., Mangalat,N., and Rhoads,J.M. (2010). Human-derived probiotic Lactobacillus reuteri strains differentially reduce intestinal inflammation. American Journal of Physiology-Gastrointestinal and Liver Physiology *299*, G1087-G1096.

Livingston,M., Loach,D., Wilson,M., Tannock,G.W., and Baird,M. (2010). Gut commensal Lactobacillus reuteri 100-23 stimulates an immunoregulatory response. Immunology and Cell Biology *88*, 99-102.

Loguercio,C., Federico,A., Tuccillo,C., Terracciano,F., D'Auria,M.V., De Simone,C., and Blanco,C.D. (2005). Beneficial effects of a probiotic VSL#3 on parameters of liver dysfunction in chronic liver diseases. Journal of Clinical Gastroenterology *39*, 540-543.

Lomax,A.R. and Calder,P.C. (2009). Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. Curr. Pharm. Des *15*, 1428-1518.

Longo,W.E., Ballantyne,G.H., Savoca,P.E., Adrian,T.E., Bilchik,A.J., and Modlin,I.M. (1991). Short-chain fatty acid release of peptide YY in the isolated rabbit distal colon. Scand. J. Gastroenterol. *26*, 442-448.

Lottenberg,A.M., Afonso,M.S., Lavrador,M.S., Machado,R.M., and Nakandakare,E.R. (2012). The role of dietary fatty acids in the pathology of metabolic syndrome. J. Nutr. Biochem. *23*, 1027-1040.

Lu,Y.C., Yeh,W.C., and Ohashi,P.S. (2008). LPS/TLR4 signal transduction pathway. Cytokine *42*, 145-151.

Lu,Y.C., Yin,L.T., Chang,W.T., and Huang,J.S. (2010). Effect of Lactobacillus reuteri GMNL-263 treatment on renal fibrosis in diabetic rats. J. Biosci. Bioeng. *110*, 709-715.

Luoto,R., Laitinen,K., Nermes,M., and Isolauri,E. (2010). Impact of maternal probiotic-supplemented dietary counselling on pregnancy outcome and prenatal and postnatal growth: a double-blind, placebo-controlled study. British Journal of Nutrition *103*, 1792-1799.

Luyer,M.D., Buurman,W.A., Hadfoune,M., Speelmans,G., Knol,J., Jacobs,J.A., Dejong,C.H., Vriesema,A.J., and Greve,J.W. (2005). Strain-specific effects of probiotics on gut barrier integrity following hemorrhagic shock. Infect. Immun. *73*, 3686-3692.

Lye,H.S., Kuan,C.Y., Ewe,J.A., Fung,W.Y., and Liong,M.T. (2009). The Improvement of Hypertension by Probiotics: Effects on Cholesterol, Diabetes, Renin, and Phytoestrogens. International Journal of Molecular Sciences *10*, 3755-3775.

Ma,X., Hua,J., and Li,Z. (2008). Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. J. Hepatol. *49*, 821-830.

Manson,J.E., Rimm,E.B., Stampfer,M.J., Colditz,G.A., Willett,W.C., Krolewski,A.S., Rosner,B., Hennekens,C.H., and Speizer,F.E. (1991). Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet *338*, 774-778.

Mantzoros,C.S., Magkos,F., Brinkoetter,M., Sienkiewicz,E., Dardeno,T.A., Kim,S.Y., Hamnvik,O.P., and Koniaris,A. (2011). Leptin in human physiology and pathophysiology. Am. J. Physiol Endocrinol. Metab *301*, E567-E584.

Mao,Y., Nobaek,S., Kasravi,B., Adawi,D., Stenram,U., Molin,G., and Jeppsson,B. (1996). The effects of Lactobacillus strains and oat fiber on methotrexate-induced enterocolitis in rats. Gastroenterology *111*, 334-344.

Mari,A., Pacini,G., Brazzale,A.R., and Ahren,B. (2005). Comparative evaluation of simple insulin sensitivity methods based on the oral glucose tolerance test. Diabetologia *48*, 748-751.

Mari,A., Pacini,G., Murphy,E., Ludvik,B., and Nolan,J.J. (2001). A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care *24*, 539-548.

Martin,G.R., Wallace,L.E., Hartmann,B., Holst,J.J., Demchyshyn,L., Toney,K., and Sigalet,D.L. (2005). Nutrient-stimulated GLP-2 release and crypt cell proliferation in experimental short bowel syndrome. Am. J. Physiol Gastrointest. Liver Physiol *288*, G431-G438.

Martinez-Rodriguez,R. and Gil,A. (2012). Nutrient-mediated modulation of incretin gene expression: a systematic review. Nutr. Hosp. *27*, 46-53.

Masur,K., Schwartz,F., Entschladen,F., Niggemann,B., and Zaenker,K.S. (2006). DPPIV inhibitors extend GLP-2 mediated tumour promoting effects on intestinal cancer cells. Regul. Pept. *137*, 147-155.

Matsuda,M. and Defronzo,R.A. (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care *22*, 1462-1470.

Matsuzaki,T., Nagata,Y., Kado,S., Uchida,K., Hashimoto,S., and Yokokura,T. (1997a). Effect of oral administration of Lactobacillus casei on alloxan-induced diabetes in mice. APMIS *105*, 637-642.

Matsuzaki,T., Nagata,Y., Kado,S., Uchida,K., Kato,I., Hashimoto,S., and Yokokura,T. (1997b). Prevention of onset in an insulin-dependent diabetes mellitus model, NOD mice, by oral feeding of Lactobacillus casei. APMIS *105*, 643-649.

Matsuzaki,T., Yamazaki,R., Hashimoto,S., and Yokokura,T. (1997c). Antidiabetic effects of an oral administration of Lactobacillus casei in a non-insulin-dependent diabetes mellitus (NIDDM) model using KK-Ay mice. Endocr. J. *44*, 357-365.

Matthews,D.R., Hosker,J.P., Rudenski,A.S., Naylor,B.A., Treacher,D.F., and Turner,R.C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia *28*, 412-419.

McCarthy,J., O'Mahony,L., O'Callaghan,L., Sheil,B., Vaughan,E.E., Fitzsimons,N., Fitzgibbon,J., O'Sullivan,G.C., Kiely,B., Collins,J.K., and Shanahan,F. (2003). Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. Gut *52*, 975-980.

MCINTYRE,N., HOLDSWORTH,C.D., and TURNER,D.S. (1964). NEW INTERPRETATION OF ORAL GLUCOSE TOLERANCE. Lancet *2*, 20-21.

MCINTYRE,N., HOLDSWORTH,C.D., and TURNER,D.S. (1965). Intestinal factors in the control of insulin secretion. J. Clin. Endocrinol. Metab *25*, 1317-1324.

Medzhitov,R. (2001). Toll-like receptors and innate immunity. Nat. Rev. Immunol. *1*, 135-145.

Medzhitov,R. (2007). Recognition of microorganisms and activation of the immune response. Nature *449*, 819-826.

Meier,C.A., Bobbioni,E., Gabay,C., Assimacopoulos-Jeannet,F., Golay,A., and Dayer,J.M. (2002). IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? J. Clin. Endocrinol. Metab *87*, 1184-1188.

Meier,J.J., Breuer,T.G., Bonadonna,R.C., Tannapfel,A., Uhl,W., Schmidt,W.E., Schrader,H., and Menge,B.A. (2012). Pancreatic diabetes manifests when beta cell area declines by approximately 65% in humans. Diabetologia *55*, 1346-1354.

Meier,J.J., Hucking,K., Holst,J.J., Deacon,C.F., Schmiegel,W.H., and Nauck,M.A. (2001). Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. Diabetes *50*, 2497-2504.

Meier,J.J., Nauck,M.A., Pott,A., Heinze,K., Goetze,O., Bulut,K., Schmidt,W.E., Gallwitz,B., and Holst,J.J. (2006). Glucagon-like peptide 2 stimulates glucagon secretion, enhances lipid absorption, and inhibits gastric acid secretion in humans. Gastroenterology *130*, 44-54.

Meigs,J.B., Cupples,L.A., and Wilson,P.W. (2000). Parental transmission of type 2 diabetes: the Framingham Offspring Study. Diabetes *49*, 2201-2207.

Menard,S., Candalh,C., Bambou,J.C., Terpend,K., Cerf-Bensussan,N., and Heyman,M. (2004). Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. Gut *53*, 821-828.

Michael,M.D., Kulkarni,R.N., Postic,C., Previs,S.F., Shulman,G.I., Magnuson,M.A., and Kahn,C.R. (2000). Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol. Cell *6*, 87-97.

Mogensen,M., Sahlin,K., Fernstrom,M., Glintborg,D., Vind,B.F., Beck-Nielsen,H., and Hojlund,K. (2007). Mitochondrial respiration is decreased in skeletal muscle of patients with type 2 diabetes. Diabetes *56*, 1592-1599.

Mojiminiyi,O.A. and Abdella,N.A. (2010). Effect of homeostasis model assessment computational method on the definition and associations of insulin resistance. Clin. Chem. Lab Med. *48*, 1629-1634.

Moran,G.W., O'Neill,C., and McLaughlin,J.T. (2012). GLP-2 enhances barrier formation and attenuates TNFalpha-induced changes in a Caco-2 cell model of the intestinal barrier. Regul. Pept. *178*, 95-101.

Morton,G.J. and Schwartz,M.W. (2011). Leptin and the central nervous system control of glucose metabolism. Physiol Rev. *91*, 389-411.

Muscelli,E., Mari,A., Natali,A., Astiarraga,B.D., Camastra,S., Frascerra,S., Holst,J.J., and Ferrannini,E. (2006). Impact of incretin hormones on beta-cell function in subjects with normal or impaired glucose tolerance. Am. J. Physiol Endocrinol. Metab *291*, E1144-E1150.

Musso,G., Gambino,R., and Cassader,M. (2010). Gut microbiota as a regulator of energy homeostasis and ectopic fat deposition: mechanisms and implications for metabolic disorders. Current Opinion in Lipidology *21*, 76-83.

Nagasumi,K., Esaki,R., Iwachidow,K., Yasuhara,Y., Ogi,K., Tanaka,H., Nakata,M., Yano,T., Shimakawa,K., Taketomi,S., Takeuchi,K., Odaka,H., and Kaisho,Y. (2009). Overexpression of GPR40 in pancreatic beta-cells augments glucose-stimulated insulin secretion and improves glucose tolerance in normal and diabetic mice. Diabetes *58*, 1067-1076.

Nauck,M., Stockmann,F., Ebert,R., and Creutzfeldt,W. (1986). Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia *29*, 46-52.

Nauck,M.A., El Ouaghlidi,A., Gabrys,B., Hucking,K., Holst,J.J., Deacon,C.F., Gallwitz,B., Schmidt,W.E., and Meier,J.J. (2004). Secretion of incretin hormones (GIP and GLP-1) and incretin effect after oral glucose in first-degree relatives of patients with type 2 diabetes. Regulatory Peptides *122*, 209-217.

Nauck,M.A., Heimesaat,M.M., Orskov,C., Holst,J.J., Ebert,R., and Creutzfeldt,W. (1993). Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J. Clin. Invest *91*, 301-307.

Nauck,M.A., Niedereichholz,U., Ettler,R., Holst,J.J., Orskov,C., Ritzel,R., and Schmiegel,W.H. (1997). Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. Am. J. Physiol *273*, E981-E988.

Newsholme,P., Keane,D., Welters,H.J., and Morgan,N.G. (2007). Life and death decisions of the pancreatic beta-cell: the role of fatty acids. Clin. Sci. (Lond) *112*, 27-42.

Nissen,L., Chingwaru,W., Sgorbati,B., Biavati,B., and Cencic,A. (2009). Gut health promoting activity of new putative probiotic/protective Lactobacillus spp. strains: a functional study in the small intestinal cell model. Int. J. Food Microbiol. *135*, 288-294.

Nolan,C.J., Damm,P., and Prentki,M. (2011). Type 2 diabetes across generations: from pathophysiology to prevention and management. Lancet *378*, 169-181.

Nowotny,B., Nowotny,P.J., Strassburger,K., and Roden,M. (2012). Precision and accuracy of blood glucose measurements using three different instruments. Diabet. Med. *29*, 260-265.

Nowotny,B., Zahiragic,L., Krog,D., Nowotny,P., Herder,C., Carstensen,M., Yoshimura ,T., Szendroedi,J., Phielix,E., Schadewaldt,P., Schloot,N., Shulman,G.I., and Roden,M. (2013). Mechnisms underlying the onset of oral lipid-induced skeletal muscle insulin resistance in humans. Diabetes.

O'Hara,A.M. and Shanahan,F. (2007). Mechanisms of action of probiotics in intestinal diseases. ScientificWorldJournal. *7*, 31-46.

Orskov,C., Holst,J.J., Khuhtsen,S., Baldissera,F.G.A., Poulsen,S.S., and Nielsen,O.V. (1986). Glucagonlike Peptides Glp-1 and Glp-2, Predicted Products of the Glucagon Gene, Are Secreted Separately from Pig Small-Intestine But Not Pancreas. Endocrinology *119*, 1467-1475.

Orskov,C., Rabenhoj,L., Wettergren,A., Kofod,H., and Holst,J.J. (1994). Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. Diabetes *43*, 535-539.

Ouwehand,A.C., Tiihonen,K., Saarinen,M., Putaala,H., and Rautonen,N. (2009). Influence of a combination of Lactobacillus acidophilus NCFM and lactitol on healthy elderly: intestinal and immune parameters. British Journal of Nutrition *101*, 367-375.

Pacini,G. (2006). The hyperbolic equilibrium between insulin sensitivity and secretion. Nutr. Metab Cardiovasc. Dis. *16 Suppl 1*, S22-S27.

Pal,S. and Radavelli-Bagatini,S. (2012). Effects of psyllium on metabolic syndrome risk factors. Obes. Rev. *13*, 1034-1047.

Parker,H.E., Reimann,F., and Gribble,F.M. (2010). Molecular mechanisms underlying nutrient-stimulated incretin secretion. Expert. Rev. Mol. Med. *12*, e1.

Parnell,J.A. and Reimer,R.A. (2009). Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. American Journal of Clinical Nutrition *89*, 1751-1759.

Parnell,J.A. and Reimer,R.A. (2012). Prebiotic fiber modulation of the gut microbiota improves risk factors for obesity and the metabolic syndrome. Gut Microbes. *3*, 29-34.

Paturi,G., Phillips,M., and Kailasapathy,K. (2008). Effect of probiotic strains Lactobacillus acidophilus LAFTI L10 and Lactobacillus paracasei LAFTI L26 on systemic immune functions and bacterial translocation in mice. J. Food Prot. *71*, 796-801.

Pendyala,S., Walker,J.M., and Holt,P.R. (2012). A high-fat diet is associated with endotoxemia that originates from the gut. Gastroenterology *142*, 1100-1101.

Perea,A., Clemente,F., Martinell,J., Villanueva-Penacarrillo,M.L., and Valverde,I. (1995). Physiological effect of glucagon in human isolated adipocytes. Horm. Metab Res. *27*, 372-375.

Pereira,M.A., Jacobs,D.R., Jr., Pins,J.J., Raatz,S.K., Gross,M.D., Slavin,J.L., and Seaquist,E.R. (2002). Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults. Am. J. Clin. Nutr. *75*, 848-855.

Pereira,M.A., Kartashov,A.I., Ebbeling,C.B., Van,H.L., Slattery,M.L., Jacobs,D.R., Jr., and Ludwig,D.S. (2005). Fast-food habits, weight gain, and insulin resistance (the CARDIA study): 15-year prospective analysis. Lancet *365*, 36-42.

Perri,F., Pastore,M.R., and Annese,V. (2005). 13C-octanoic acid breath test for measuring gastric emptying of solids. Eur. Rev. Med. Pharmacol. Sci. *9*, 3-8.

Petersen,K.F., Dufour,S., Savage,D.B., Bilz,S., Solomon,G., Yonemitsu,S., Cline,G.W., Befroy,D., Zemany,L., Kahn,B.B., Papademetris,X., Rothman,D.L., and Shulman,G.I. (2007). The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc. Natl. Acad. Sci. U. S. A *104*, 12587-12594.

Pfleger,C., Kaas,A., Hansen,L., Alizadeh,B., Hougaard,P., Holl,R., Kolb,H., Roep,B.O., Mortensen,H.B., and Schloot,N.C. (2008a). Relation of circulating concentrations of chemokine receptor CCR5 ligands to C-peptide, proinsulin and HbA1c and disease progression in type 1 diabetes. Clinical Immunology *128*, 57-65.

Pfleger,C., Mortensen,H.B., Hansen,L., Herder,C., Roep,B.O., Hoey,H., Aanstoot,H.J., Kocova,M., and Schloot,N.C. (2008b). Association of IL-1ra and adiponectin with C-peptide and remission in patients with type 1 diabetes. Diabetes *57*, 929-937.

Pham,M.N., Hawa,M.I., Pfleger,C., Roden,M., Schernthaner,G., Pozzilli,P., Buzzetti,R., Scherbaum,W., Seissler,J., Kolb,H., Hunter,S., Leslie,R.D.G., and Schloot,N.C. (2011). Pro- and anti-inflammatory cytokines in latent autoimmune diabetes in adults, type 1 and type 2 diabetes patients: Action LADA 4. Diabetologia *54*, 1630-1638.

Pham,M.N., Kolb,H., Mandrup-Poulsen,T., Battelino,T., Ludvigsson,J., Pozzilli,P., Roden,M., and Schloot,N.C. (2013). Serum adipokines as biomarkers of beta-cell function in patients with type 1 diabetes: positive association with leptin and resistin and negative association with adiponectin. Diabetes Metab Res. Rev. *29*, 166-170.

Phielix,E., Meex,R., Ouwens,D.M., Sparks,L., Hoeks,J., Schaart,G., Moonen-Kornips,E., Hesselink,M.K., and Schrauwen,P. (2012). High oxidative capacity due to chronic exercise training attenuates lipid-induced insulin resistance. Diabetes *61*, 2472-2478.

Phielix,E., Szendroedi,J., and Roden,M. (2011). Mitochondrial function and insulin resistance during aging: a mini-review. Gerontology *57*, 387-396.

Pierce,M., Keen,H., and Bradley,C. (1995). Risk of diabetes in offspring of parents with non-insulin-dependent diabetes. Diabet. Med. *12*, 6-13.

Pipeleers,D.G., Schuit,F.C., in't Veld,P.A., Maes,E., Hooghe-Peters,E.L., Van de Winkel,M., and Gepts,W. (1985). Interplay of nutrients and hormones in the regulation of insulin release. Endocrinology *117*, 824-833.

Plaisancie,P., Dumoulin,V., Chayvialle,J.A., and Cuber,J.C. (1995). Luminal glucagon-like peptide-1(7-36) amide-releasing factors in the isolated vascularly perfused rat colon. J. Endocrinol. *145*, 521-526.

Poulsen,P., Grunnet,L.G., Pilgaard,K., Storgaard,H., Alibegovic,A., Sonne,M.P., Carstensen,B., Beck-Nielsen,H., and Vaag,A. (2009). Increased risk of type 2 diabetes in elderly twins. Diabetes *58*, 1350-1355.

Poulsen,P., Kyvik,K.O., Vaag,A., and Beck-Nielsen,H. (1999). Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance--a population-based twin study. Diabetologia *42*, 139-145.

Pradhan,A.D., Manson,J.E., Rifai,N., Buring,J.E., and Ridker,P.M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA *286*, 327-334.

Pratipanawatr,W., Pratipanawatr,T., Cusi,K., Berria,R., Adams,J.M., Jenkinson,C.P., Maezono,K., Defronzo,R.A., and Mandarino,L.J. (2001). Skeletal muscle insulin resistance in normoglycemic

subjects with a strong family history of type 2 diabetes is associated with decreased insulin-stimulated insulin receptor substrate-1 tyrosine phosphorylation. Diabetes *50*, 2572-2578.

Pratley,R.E. (1998). Gene-environment interactions in the pathogenesis of type 2 diabetes mellitus: lessons learned from the Pima Indians. Proc. Nutr. Soc. *57*, 175-181.

Qi,L., Rimm,E., Liu,S., Rifai,N., and Hu,F.B. (2005). Dietary glycemic index, glycemic load, cereal fiber, and plasma adiponectin concentration in diabetic men. Diabetes Care *28*, 1022-1028.

Qin,J., Li,Y., Cai,Z., Li,S., Zhu,J., Zhang,F., Liang,S., Zhang,W., Guan,Y., Shen,D., Peng,Y., Zhang,D., Jie,Z., Wu,W., Qin,Y., Xue,W., Li,J., Han,L., Lu,D., Wu,P., Dai,Y., Sun,X., Li,Z., Tang,A., Zhong,S., Li,X., Chen,W., Xu,R., Wang,M., Feng,Q., Gong,M., Yu,J., Zhang,Y., Zhang,M., Hansen,T., Sanchez,G., Raes,J., Falony,G., Okuda,S., Almeida,M., LeChatelier,E., Renault,P., Pons,N., Batto,J.M., Zhang,Z., Chen,H., Yang,R., Zheng,W., Li,S., Yang,H., Wang,J., Ehrlich,S.D., Nielsen,R., Pedersen,O., Kristiansen,K., and Wang,J. (2012). A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature *490*, 55-60.

Quddusi,S., Vahl,T.P., Hanson,K., Prigeon,R.L., and D'Alessio,D.A. (2003). Differential effects of acute and extended infusions of glucagon-like peptide-1 on first- and second-phase insulin secretion in diabetic and nondiabetic humans. Diabetes Care *26*, 791-798.

Rabol,R., Petersen,K.F., Dufour,S., Flannery,C., and Shulman,G.I. (2011). Reversal of muscle insulin resistance with exercise reduces postprandial hepatic de novo lipogenesis in insulin resistant individuals. Proc. Natl. Acad. Sci. U. S. A *108*, 13705-13709.

Ramnani,P., Chitarrari,R., Tuohy,K., Grant,J., Hotchkiss,S., Philp,K., Campbell,R., Gill,C., and Rowland,I. (2012). In vitro fermentation and prebiotic potential of novel low molecular weight polysaccharides derived from agar and alginate seaweeds. Anaerobe. *18*, 1-6.

Reaven,G.M. (1988). Banting lecture 1988. Role of insulin resistance in human disease. Diabetes *37*, 1595-1607.

Reimer,R.A., Maurer,A.D., Eller,L.K., Hallam,M.C., Shaykhutdinov,R., Vogel,H.J., and Weljie,A.M. (2012). Satiety hormone and metabolomic response to an intermittent high energy diet differs in rats consuming long-term diets high in protein or prebiotic fiber. J. Proteome. Res. *11*, 4065-4074.

Ristow,M., Zarse,K., Oberbach,A., Kloting,N., Birringer,M., Kiehntopf,M., Stumvoll,M., Kahn,C.R., and Bluher,M. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. Proceedings of the National Academy of Sciences of the United States of America *106*, 8665-8670.

Roberfroid,M., Gibson,G.R., Hoyles,L., McCartney,A.L., Rastall,R., Rowland,I., Wolvers,D., Watzl,B., Szajewska,H., Stahl,B., Guarner,F., Respondek,F., Whelan,K., Coxam,V., Davicco,M.J., Leotoing,L., Wittrant,Y., Delzenne,N.M., Cani,P.D., Neyrinck,A.M., and Meheust,A. (2010). Prebiotic effects: metabolic and health benefits. Br. J. Nutr. *104 Suppl 2*, S1-63.

Robertson,M.D., Currie,J.M., Morgan,L.M., Jewell,D.P., and Frayn,K.N. (2003). Prior short-term consumption of resistant starch enhances postprandial insulin sensitivity in healthy subjects. Diabetologia *46*, 659-665.

Rodes,L., Khan,A., Paul,A., Coussa-Charley,M., Marinescu,D., Tomaro-Duchesneau,C., Shao,W., Kahouli,I., and Prakash,S. (2013). Effect of probiotics lactobacillus and bifidobacterium on gut-derived lipopolysaccharides and inflammatory cytokines: an in vitro study using a human colonic microbiota model. J. Microbiol. Biotechnol. *23*, 518-526.

Roelofsen,H., Priebe,M.G., and Vonk,R.J. (2010). The interaction of short-chain fatty acids with adipose tissue: relevance for prevention of type 2 diabetes. Benef. Microbes. *1*, 433-437.

Roglic,G., Unwin,N., Bennett,P.H., Mathers,C., Tuomilehto,J., Nag,S., Connolly,V., and King,H. (2005). The burden of mortality attributable to diabetes: realistic estimates for the year 2000. Diabetes Care *28*, 2130-2135.

Rosen,E.D. and Spiegelman,B.M. (2006). Adipocytes as regulators of energy balance and glucose homeostasis. Nature *444*, 847-853.

Rowland,K.J. and Brubaker,P.L. (2008). Life in the crypt: a role for glucagon-like peptide-2? Mol. Cell Endocrinol. *288*, 63-70.

Ruan,H. and Lodish,H.F. (2003). Insulin resistance in adipose tissue: direct and indirect effects of tumor necrosis factor-alpha. Cytokine Growth Factor Rev. *14*, 447-455.

Ruiz-Palacios,G., Tuz,F., Arteaga,F., Guerrero,M.L., Dohnalek,M., and Hilty,M. (1992). Tolerance and fecal colonization with Lactobacillus reuteri in children fed a beverage with a mixture of Lactobacillus spp. Pediatr Res *39*, 1090.

Saad,M.F., Knowler,W.C., Pettitt,D.J., Nelson,R.G., Mott,D.M., and Bennett,P.H. (1988). The natural history of impaired glucose tolerance in the Pima Indians. N. Engl. J. Med. *319*, 1500-1506.

Samaras,K., Botelho,N.K., Chisholm,D.J., and Lord,R.V. (2010). Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. Obesity. (Silver. Spring) *18*, 884-889.

Samsom,M., Bharucha,A., Gerich,J.E., Herrmann,K., Limmer,J., Linke,R., Maggs,D., Schirra,J., Vella,A., Worle,H.J., and Goke,B. (2009). Diabetes mellitus and gastric emptying: questions and issues in clinical practice. Diabetes Metab Res Rev. *25*, 502-514.

Samuel,V.T., Petersen,K.F., and Shulman,G.I. (2010). Lipid-induced insulin resistance: unravelling the mechanism. Lancet *375*, 2267-2277.

Samuel,V.T. and Shulman,G.I. (2012). Mechanisms for insulin resistance: common threads and missing links. Cell *148*, 852-871.

Sapone,A., de,M.L., Pietzak,M., Clemente,M.G., Tripathi,A., Cucca,F., Lampis,R., Kryszak,D., Carteni,M., Generoso,M., Iafusco,D., Prisco,F., Laghi,F., Riegler,G., Carratu,R., Counts,D., and Fasano,A. (2006). Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. Diabetes *55*, 1443-1449.

Savage,D.B., Petersen,K.F., and Shulman,G.I. (2007). Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev. *87*, 507-520.

Savino,F., Pelle,E., Palumeri,E., Oggero,R., and Miniero,R. (2007). Lactobacillus reuteri (American type culture collection strain 55730) versus simethicone in the treatment of infantile colic: A prospective randomized study. Pediatrics *119*, E124-E130.

Scaldaferri,F., Pizzoferrato,M., Gerardi,V., Lopetuso,L., and Gasbarrini,A. (2012). The gut barrier: new acquisitions and therapeutic approaches. J. Clin. Gastroenterol. *46 Suppl*, S12-S17.

Schadewaldt,P., Nowotny,B., Straßburger,K., Kotzka,J., and Roden,M. (2013). Indirect calorimetry in humans: a post-calorimetric evaluation procedure for correction of metabolic monitor variability. American Journal of Clinical Nutrition.

Schenk,S., Davidson,C.J., Zderic,T.W., Byerley,L.O., and Coyle,E.F. (2003). Different glycemic indexes of breakfast cereals are not due to glucose entry into blood but to glucose removal by tissue. Am. J. Clin. Nutr. *78*, 742-748.

Schloot,N.C., Hanifi-Moghaddam,P., Aabenhus-Andersen,N., Alizadeh,B.Z., Saha,M.T., Knip,M., Devendra,D., Wilkin,T., Bonifacio,E., Roep,B.O., Kolb,H., and Mandrup-Poulsen,T. (2007). Association of immune mediators at diagnosis of Type 1 diabetes with later clinical remission. Diabetic Medicine *24*, 512-520.

Segal,K.R., Van,L.M., Fitzgerald,P.I., Hodgdon,J.A., and Van Itallie,T.B. (1988). Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study. Am. J. Clin. Nutr. *47*, 7-14.

Seppo,L., Jauhiainen,T., Poussa,T., and Korpela,R. (2003). A fermented milk high in bioactive peptides has a blood pressure-lowering effect in hypertensive subjects. American Journal of Clinical Nutrition *77*, 326-330.

Shaw,J.E., Sicree,R.A., and Zimmet,P.Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract. *87*, 4-14.

Shen,L., Keenan,M.J., Raggio,A., Williams,C., and Martin,R.J. (2011). Dietary-resistant starch improves maternal glycemic control in Goto-Kakizaki rat. Mol. Nutr. Food Res. *55*, 1499-1508.

Shornikova,A.V., Casas,I.A., Isolauri,E., Mykkanen,H., and Vesikari,T. (1997a). Lactobacillus reuteri as a therapeutic agent in acute diarrhea in young children. Journal of Pediatric Gastroenterology and Nutrition *24*, 399-404.

Shornikova,A.V., Casas,I.A., Mykkanen,H., Salo,E., and Vesikari,T. (1997b). Bacteriotherapy with Lactobacillus reuteri in rotavirus gastroenteritis. Pediatric Infectious Disease Journal *16*, 1103-1107.

Simon,M.C., Bilan,S., Nowotny,B., Dickhaus,T., Burkart,V., and Schloot,N.C. (2013). Fatty acids modulate cytokine and chemokine secretion of stimulated human whole blood cultures in diabetes. Clin. Exp. Immunol. *172*, 383-393.

Sinkiewicz, G. and Nordström, E. A. Occurrence of Lactobacillus reuteri, lactobacilli and bifidobacteria in human breast milk. Pediatr Res [58], 415. 2005.
Ref Type: Abstract

Sipola,M., Finckenberg,P., Korpela,R., Vapaatalo,H., and Nurminen,M.L. (2002). Effect of long-term intake of milk products on blood pressure in hypertensive rats. Journal of Dairy Research *69*, 103-111.

Sladek,R., Rocheleau,G., Rung,J., Dina,C., Shen,L., Serre,D., Boutin,P., Vincent,D., Belisle,A., Hadjadj,S., Balkau,B., Heude,B., Charpentier,G., Hudson,T.J., Montpetit,A., Pshezhetsky,A.V., Prentki,M., Posner,B.I., Balding,D.J., Meyre,D., Polychronakos,C., and Froguel,P. (2007). A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature *445*, 881-885.

Solga,S.F., Buckley,G., Clark,J.M., Horska,A., and Diehl,A.M. (2008). The Effect of a Probiotic on Hepatic Steatosis. Journal of Clinical Gastroenterology *42*, 1117-1119.

Sommer,F. and Backhed,F. (2013). The gut microbiota--masters of host development and physiology. Nat. Rev. Microbiol. *11*, 227-238.

Soodini,G.R. (2004). Adiponectin and leptin in relation to insulin sensitivity. Metab Syndr. Relat Disord. *2*, 114-123.

Spranger,J., Kroke,A., Mohlig,M., Hoffmann,K., Bergmann,M.M., Ristow,M., Boeing,H., and Pfeiffer,A.F. (2003). Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes *52*, 812-817.

Stumvoll,M., Goldstein,B.J., and van Haeften,T.W. (2005). Type 2 diabetes: principles of pathogenesis and therapy. Lancet *365*, 1333-1346.

Sun,K., Xie,C., Xu,D., Yang,X., Tang,J., and Ji,X. (2013). Lactobacillus isolates from healthy volunteers exert immunomodulatory effects on activated peripheral blood mononuclear cells. J. Biomed. Res. *27*, 116-126.

Szendroedi,J., Phielix,E., and Roden,M. (2012). The role of mitochondria in insulin resistance and type 2 diabetes mellitus. Nature Reviews Endocrinology *8*, 92-103.

Szendroedi,J. and Roden,M. (2009). Ectopic lipids and organ function. Curr. Opin. Lipidol. *20*, 50-56.

Szendroedi,J., Schmid,A.I., Chmelik,M., Krssak,M., Nowotny,P., Prikoszovich,T., Kautzky-Willer,A., Wolzt,M., Waldhausl,W., and Roden,M. (2011). Skeletal muscle phosphodiester content relates to body mass and glycemic control. PLoS. One. *6*, e21846.

Tabak,A.G., Jokela,M., Akbaraly,T.N., Brunner,E.J., Kivimaki,M., and Witte,D.R. (2009). Trajectories of glycaemia, insulin sensitivity, and insulin secretion before diagnosis of type 2 diabetes: an analysis from the Whitehall II study. Lancet *373*, 2215-2221.

Tabuchi,M., Ozaki,M., Tamura,A., Yamada,N., Ishida,T., Hosoda,M., and Hosono,A. (2003). Antidiabetic effect of Lactobacillus GG in streptozotocin-induced diabetic rats. Biosci. Biotechnol. Biochem. *67*, 1421-1424.

Takamura,T., Misu,H., Ota,T., and Kaneko,S. (2012). Fatty liver as a consequence and cause of insulin resistance: lessons from type 2 diabetic liver. Endocr. J. *59*, 745-763.

Takeda,K. and Akira,S. (2004). TLR signaling pathways. Semin. Immunol. *16*, 3-9.

Tan,T.M., Field,B.C., McCullough,K.A., Troke,R.C., Chambers,E.S., Salem,V., Gonzalez,M.J., Baynes,K.C., De,S.A., Viardot,A., Alsafi,A., Frost,G.S., Ghatei,M.A., and Bloom,S.R. (2013). Coadministration of glucagon-like Peptide-1 during glucagon infusion in humans results in increased energy expenditure and amelioration of hyperglycemia. Diabetes *62*, 1131-1138.

Tanaka,T., Katsuma,S., Adachi,T., Koshimizu,T.A., Hirasawa,A., and Tsujimoto,G. (2008). Free fatty acids induce cholecystokinin secretion through GPR120. Naunyn Schmiedebergs Arch. Pharmacol. *377*, 523-527.

Tappenden,K.A., Albin,D.M., Bartholome,A.L., and Mangian,H.F. (2003). Glucagon-like peptide-2 and short-chain fatty acids: a new twist to an old story. J. Nutr. *133*, 3717-3720.

Tarini,J. and Wolever,T.M.S. (2010). The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition et Metabolisme *35*, 9-16.

Tattersal,R.B. and Fajans,S.S. (1975). Prevalence of diabetes and glucose intolerance in 199 offspring of thirty-seven conjugal diabetic parents. Diabetes *24*, 452-462.

Tazoe,H., Otomo,Y., Kaji,I., Tanaka,R., Karaki,S.I., and Kuwahara,A. (2008). Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. J. Physiol Pharmacol. *59 Suppl 2*, 251-262.

Teixeira,T.F., Collado,M.C., Ferreira,C.L., Bressan,J., and Peluzio,M.C. (2012). Potential mechanisms for the emerging link between obesity and increased intestinal permeability. Nutr. Res. *32*, 637-647.

Thomas,T. and Pfeiffer,A.F. (2012). Foods for the prevention of diabetes: how do they work? Diabetes Metab Res. Rev. *28*, 25-49.

Thomaseth,K., Kautzky-Willer,A., Ludvik,B., Prager,R., and Pacini,G. (1996). Integrated mathematical model to assess beta-cell activity during the oral glucose test. Am. J. Physiol *270*, E522-E531.

Toft-Nielsen,M.B., Damholt,M.B., Madsbad,S., Hilsted,L.M., Hughes,T.E., Michelsen,B.K., and Holst,J.J. (2001). Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. Journal of Clinical Endocrinology & Metabolism *86*, 3717-3723.

Tolhurst,G., Heffron,H., Lam,Y.S., Parker,H.E., Habib,A.M., Diakogiannaki,E., Cameron,J., Grosse,J., Reimann,F., and Gribble,F.M. (2012). Short-Chain Fatty Acids Stimulate Glucagon-Like Peptide-1 Secretion via the G-Protein-Coupled Receptor FFAR2. Diabetes *61*, 364-371.

Tremaroli,V. and Backhed,F. (2012). Functional interactions between the gut microbiota and host metabolism. Nature *489*, 242-249.

Turnbaugh,P.J., Ley,R.E., Mahowald,M.A., Magrini,V., Mardis,E.R., and Gordon,J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature *444*, 1027-1031.

Unger,R.H. (2002). Lipotoxic diseases. Annu. Rev. Med. *53*, 319-336.

Vaag,A.A., Holst,J.J., Volund,A., and Beck-Nielsen,H.B. (1996). Gut incretin hormones in identical twins discordant for non-insulin-dependent diabetes mellitus (NIDDM)--evidence for decreased glucagon-like peptide 1 secretion during oral glucose ingestion in NIDDM twins. Eur. J. Endocrinol. *135*, 425-432.

Vajro,P., Paolella,G., and Fasano,A. (2013). Microbiota And Gut-Liver Axis: A Mini-Review On Their Influences On Obesity And Obesity Related Liver Disease. J. Pediatr. Gastroenterol. Nutr.

Van,N.E., Vrieze,A., Nieuwdorp,M., Fuentes,S., Zoetendal,E.G., de Vos,W.M., Visser,C.E., Kuijper,E.J., Bartelsman,J.F., Tijssen,J.G., Speelman,P., Dijkgraaf,M.G., and Keller,J.J. (2013). Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile. N. Engl. J. Med.

Vettor,R., Granzotto,M., De,S.D., Trevellin,E., Rossato,M., Farina,M.G., Milan,G., Pilon,C., Nigro,A., Federspil,G., Vigneri,R., Vitiello,L., Rizzuto,R., Baratta,R., and Frittitta,L. (2008). Loss-of-function mutation of the GPR40 gene associates with abnormal stimulated insulin secretion by acting on intracellular calcium mobilization. J. Clin. Endocrinol. Metab *93*, 3541-3550.

Vilsboll,T., Krarup,T., Deacon,C.F., Madsbad,S., and Holst,J.J. (2001). Reduced postprandial concentrations of intact biologically active gluccagon-like peptide 1 in type 2 diabetic patients. Diabetes *50*, 609-613.

Vilsboll,T., Krarup,T., Madsbad,S., and Holst,J.J. (2003a). Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. Regul. Pept. *114*, 115-121.

Vilsboll,T., Krarup,T., Sonne,J., Madsbad,S., Volund,A., Juul,A.G., and Holst,J.J. (2003b). Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. J. Clin. Endocrinol. Metab *88*, 2706-2713.

Vrieze,A., Van,N.E., Holleman,F., Salojarvi,J., Kootte,R.S., Bartelsman,J.F., Dallinga-Thie,G.M., Ackermans,M.T., Serlie,M.J., Oozeer,R., Derrien,M., Druesne,A., Van Hylckama Vlieg,J.E., Bloks,V.W., Groen,A.K., Heilig,H.G., Zoetendal,E.G., Stroes,E.S., de Vos,W.M., Hoekstra,J.B., and Nieuwdorp,M. (2012). Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology *143*, 913-916.

Wagnerberger,S., Spruss,A., Kanuri,G., Stahl,C., Schroder,M., Vetter,W., Bischoff,S.C., and Bergheim,I. (2012). Lactobaccilus casei Shirota protects from fructose-induced liver steatosis: A mouse model. J. Nutr. Biochem.

Wallace,T.M., Levy,J.C., and Matthews,D.R. (2004). Use and abuse of HOMA modeling. Diabetes Care *27*, 1487-1495.

Wallace,T.M. and Matthews,D.R. (2002). The assessment of insulin resistance in man. Diabet. Med. *19*, 527-534.

Weickert,M.O., Loeffelholz,C.V., Roden,M., Chandramouli,V., Brehm,A., Nowotny,P., Osterhoff,M.A., Isken,F., Spranger,J., Landau,B.R., Pfeiffer,A.F., and Mohlig,M. (2007). A Thr94Ala mutation in human liver fatty acid-binding protein contributes to reduced hepatic glycogenolysis and blunted elevation of plasma glucose levels in lipid-exposed subjects. Am. J. Physiol Endocrinol. Metab *293*, E1078-E1084.

Weickert,M.O., Mohlig,M., Koebnick,C., Holst,J.J., Namsolleck,P., Ristow,M., Osterhoff,M., Rochlitz,H., Rudovich,N., Spranger,J., and Pfeiffer,A.F. (2005). Impact of cereal fibre on glucose-regulating factors. Diabetologia *48*, 2343-2353.

Weickert,M.O., Mohlig,M., Schofl,C., Arafat,A.M., Otto,B., Viehoff,H., Koebnick,C., Kohl,A., Spranger,J., and Pfeiffer,A.F. (2006a). Cereal fiber improves whole-body insulin sensitivity in overweight and obese women. Diabetes Care *29*, 775-780.

Weickert,M.O., Spranger,J., Holst,J.J., Otto,B., Koebnick,C., Mohlig,M., and Pfeiffer,A.F. (2006b). Wheat-fibre-induced changes of postprandial peptide YY and ghrelin responses are not associated with acute alterations of satiety. Br. J. Nutr. *96*, 795-798.

Weiss,R., D'Adamo,E., Santoro,N., Hershkop,K., and Caprio,S. (2011). Basal alpha-cell up-regulation in obese insulin-resistant adolescents. J. Clin. Endocrinol. Metab *96*, 91-97.

Weizman,Z., Asli,G., and Alsheikh,A. (2005). Effect of a probiotic infant formula on infections in child care centers: Comparison of two probiotic agents. Pediatrics *115*, 5-9.

Wellen,K.E. and Hotamisligil,G.S. (2005). Inflammation, stress, and diabetes. J. Clin. Invest *115*, 1111-1119.

Wettergren,A., Schjoldager,B., Mortensen,P.E., Myhre,J., Christiansen,J., and Holst,J.J. (1993). Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. Dig. Dis. Sci. *38*, 665-673.

Weyer,C., Tataranni,P.A., Bogardus,C., and Pratley,R.E. (2001). Insulin resistance and insulin secretory dysfunction are independent predictors of worsening of glucose tolerance during each stage of type 2 diabetes development. Diabetes Care *24*, 89-94.

Wild,S., Roglic,G., Green,A., Sicree,R., and King,H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care *27*, 1047-1053.

Woerle,H.J., Carneiro,L., Derani,A., Goke,B., and Schirra,J. (2012). The role of endogenous incretin secretion as amplifier of glucose-stimulated insulin secretion in healthy subjects and patients with type 2 diabetes. Diabetes *61*, 2349-2358.

Wolf,B.W., Garleb,K.A., Ataya,D.G., and Casas,I.A. (1995). Safety and tolerance of Lactobacillus reuteri in healthy adult male subjects. Microbial Ecol Health Dis 41-50.

Wolf,B.W., Wheeler,K.B., Ataya,D.G., and Garleb,K.A. (1998). Safety and tolerance of Lactobacillus reuteri supplementation to a population infected with the human immunodeficiency virus. Food Chem. Toxicol. *36*, 1085-1094.

Wong,V.S. and Brubaker,P.L. (2006). From cradle to grave: pancreatic beta-cell mass and glucagon-like peptide-1. Minerva Endocrinol. *31*, 107-124.

Xie,L., O'Reilly,C.P., Chapes,S.K., and Mora,S. (2008). Adiponectin and leptin are secreted through distinct trafficking pathways in adipocytes. Biochim. Biophys. Acta *1782*, 99-108.

Yadav,H., Jain,S., and Sinha,P.R. (2007). Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. Nutrition *23*, 62-68.

Yadav,H., Jain,S., and Sinha,P.R. (2008). Oral administration of dahi containing probiotic Lactobacillus acidophilus and Lactobacillus casei delayed the progression of streptozotocin-induced diabetes in rats. J. Dairy Res. *75*, 189-195.

Yang,S., Lin,S., Kelen,G.D., Quinn,T.C., Dick,J.D., Gaydos,C.A., and Rothman,R.E. (2002). Quantitative multiprobe PCR assay for simultaneous detection and identification to species level of bacterial pathogens. Journal of Clinical Microbiology *40*, 3449-3454.

Ziegler,D., Schadewaldt,P., Mirza,A.P., Piolot,R., Schommartz,B., Reinhardt,M., Vosberg,H., Brosicke,H., and Gries,F.A. (1996). [C-13]Octanoic acid breath test for non-invasive assessment of gastric emptying in diabetic patients: Validation and relationship to gastric symptoms and cardiovascular autonomic function. Diabetologia *39*, 823-830.

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