Hepatic steatosis : metabolic consequences

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Chapter 5

Endogenous IL-10 Protects Against Hepatic Steatosis, but Does Not Improve Insulin Sensitivity During High Fat Feeding in Mice

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Abstract

Several studies have demonstrated an association in humans between plasma levels or production capacity of the anti-inflammatory cytokine IL-10, and insulin sensitivity. The aim of our study was to investigate the protective role of endogenous IL-10 availability in the development of diet-induced insulin resistance. We compared parameters of glucose and lipid metabolism between IL-$10^{-/-}$ mice and wild type (wt) mice fed a high fat diet for 6 weeks. This diet has previously been shown to induce steatosis and insulin resistance. After 6 weeks on the high fat diet no differences in bodyweight, basal metabolism (measured by indirect calorimetry) and plasma levels of glucose, triglycerides (TG) or cholesterol were observed between IL-$10^{-/-}$ and wt mice. Nonetheless, in IL-$10^{-/-}$ mice plasma fatty acid levels were 75% increased compared to wt mice after overnight fasting ($P < 0.05$). In addition, hepatic TG content was 54% increased in IL-$10^{-/-}$ mice ($P < 0.05$). During a hyperinsulinemic euglycemic clamp no differences were observed in whole-body or hepatic insulin sensitivity between both groups.

We conclude that basal IL-10 production protects against hepatic steatosis, but does not improve hepatic or whole-body insulin sensitivity, during high fat feeding.

Introduction

In epidemiological studies insulin resistance is associated with chronic low-grade inflammation.[1] This is reflected in associations between the degree of insulin sensitivity and plasma levels of several cytokines, such as tumor necrosis factor (TNF)α and interleukin-(IL)6.[2,3] In addition, administration of exogenous TNFα and IL-6 induces insulin resistance *in vivo*.[4,5] Conversely, IL-6 depletion improves hepatic insulin action in an animal model of obesity.[6]

IL-10 is a potent anti-inflammatory cytokine, which is produced by T-cells, B-cells, monocytes and macrophages and plays a crucial role in the innate immune system.[7,8] IL-10 potently inhibits the production of pro-inflammatory cytokines, including TNFα and IL-6.[9] Several lines of evidence point to a beneficial effect of IL-10 on insulin sensitivity. A recent epidemiological study showed a positive correlation between IL-10 levels and insulin sensitivity in healthy subjects.[10] In the Leiden 85-plus study the IL-10 production capacity of whole blood was investigated using lipopolysaccharide as a stimulus. The IL-10 production capacity was found to be inversely associated with blood glucose and HBA1c levels.[11] Finally, administration of IL-10 in mice prevented IL-6–induced defects in hepatic insulin action and signalling activity.[12] Although these studies suggest a potentially beneficial role of IL-10 in insulin resistant conditions, the beneficial role of endogenous IL-10 secretion in insulin resistant states has not been proven.

To determine whether endogenous IL-10 production can protect against diet-induced insulin resistance, we compared metabolic characteristics of IL-10$^{-/-}$ mice and wild type (wt) control mice. We fed the mice a high fat diet for 6 weeks and subsequently analyzed parameters of lipid and glucose metabolism. Previous studies have documented, that high fat feeding induces accumulation of TG in the liver and hepatic insulin resistance.[13] We phenotyped the interaction between genotypes and diet by using the metabolic cages and by assessing insulin sensitivity with the hyperinsulinemic euglycemic clamp method. Our data indicate that, in contrast to our expectations, basal IL-10 production protects against hepatic steatosis during high fat feeding, but does not improve hepatic or whole-body insulin sensitivity.

Materials and Methods

Animals

Ten weeks old male C57Bl6/J mice (wt) and IL-10$^{-/-}$ mice on the same background were purchased from Charles River (Maastricht, Netherlands). Mice had free access to water and a normal chow diet (Technilab BMI, Someren, Netherlands) until 12 weeks of age. Subsequently, mice were fed a high fat diet for 6 weeks (40% of calories from bovine lard; Hope Farms, Woerden, Netherlands). A previous study showed a 2.5-fold increased liver lipid content on this high fat diet with a concurrent decrease in hepatic insulin sensitivity.[13] Mice were weighed every week and at t=0 and after 6 weeks on the high fat diet a blood sample was taken to determine plasma triglyceride (TG), cholesterol and glucose levels. Principles of laboratory animal care were followed and the animal ethics committee of our institute approved all animal experiments.

Plasma lipid and glucose analysis

In all experiments, tail vein blood was collected into chilled paraoxon-coated capillary tubes to prevent *in vitro* lipolysis.[14] These tubes were placed on ice and immediately centrifuged at 4°C. Plasma was isolated, snap-frozen in liquid nitrogen and stored at –20°C until analysis. The levels of plasma TG, total cholesterol, free fatty acids (FA) and glucose were determined enzymatically using commercially available kits and standards (#310-A Sigma GPO-Trinder kit, St. Louis, MA, USA; CHOL MPR3, Boehringer, Mannheim, Germany; #315 Sigma NEFA-C kit, St. Louis, MA, USA; Hexokinase method, Instruchemie, Netherlands).

Metabolic cages

After 6 weeks on the high fat diet basal metabolism in the IL-10$^{-/-}$ and wt mice was studied using the Comprehensive Laboratory Animal Monitoring System (CLAMS; Columbus Instruments, Columbus, USA). Metabolic rates were measured using an eight-chamber open-circuit system. Animals were maintained at approximately 24°C under a 12 h light/dark cycle. Food and water were freely available. The mice were housed individually in plexiglass cages through which 0.6 L of air was passed per min. Each chamber was sampled for 45 seconds at 7 min intervals for a 24 h period. The O_2 and CO_2 content of the exhaust air was compared to the O_2 and CO_2 content

of the standardized sample air. Before the start of the actual 24 h measurements mice were weighed and acclimatized to the cages for 24 h.

Hyperinsulinemic euglycemic clamp experiments

After 6 weeks on the high fat diet, clamp experiments were performed as described previously[15,16] after an overnight fast. Animals were anaesthetized by intraperitoneal injection with a combination of 6.25 mg/kg acetylpromazine (Sanofi Santé Nutrition Animale, Libourne Cedex, France) 6.25 mg/kg midazolam (Roche, Mijdrecht, Netherlands) and 0.3125 mg/kg fentanyl (Janssen-Cilag, Tilburg, Netherlands). An infusion needle was placed into the tail vein. After 45 min infusion of D-[3-^{3}H]glucose at a rate of 0.8 µCi/h (specific activity: 620 GBq/mmol, Amersham, Little Chalfont, UK) to achieve steady state levels, basal parameters were determined with 15 min intervals. Thereafter a bolus of insulin (4.5 mU, Actrapid, Novo Nordisk, Chartres, France) was administered and the hyperinsulinemic clamp was started. Insulin was infused at a constant rate of 6.8 mU/h and D-[3-^{3}H]glucose was infused at a rate of 0.8 µCi/h. A variable infusion of 12.5% D-glucose (in PBS) was also started to maintain blood glucose at approximately 7 mM. Blood glucose was measured with the FreeStyle hand glucose measurer (Therasense, Disetronic Medical Systems, Vianen, Netherlands) every 10 min to monitor glucose levels and adjust the glucose pump. After reaching steady state, blood samples were taken at 10 min time intervals during 30 min to determine steady state levels of [^{3}H]glucose. After the last blood sample mice were sacrificed by cervical dislocation and the organs were dissected. An average clamp experiment took approximately 3 h and anaesthesia was maintained throughout the procedure.

Analysis of clamp samples

Plasma insulin concentrations were measured by ELISA (ALPCO Diagnostics, Windham, NH, USA). To measure plasma [^{3}H]glucose trichloroacetic acid (final concentration 2%) was added to 7.5 µL plasma to precipitate proteins using centrifugation. The supernatant was dried to remove water and resuspended in milliQ. The samples were counted using scintillation counting (Packard Instruments, Dowers Grove, IL, USA).

Calculations

The glucose turnover rate (μmol·min^{-1}·kg^{-1}) was calculated during the basal period and under steady-state clamp conditions as the rate of tracer infusion (dpm/min) divided by the plasma specific activity of [^{3}H]glucose (dpm/μmol). The ratio was corrected for body weight. The hyperinsulinemic hepatic glucose production (HGP) was calculated as the difference between the tracer-derived rate of glucose appearance and the glucose infusion rate.

Determination of Akt phosphorylation in liver samples

To investigate hepatic insulin signalling, liver samples (100 mg) from clamped mice (n=4-5 mice/group) were homogenized in a buffer containing: 30 mM Tris, 2.5 mM EDTA, 150 mM NaCl, 0.5 mM Na_3VO_4, 5 mM NaF, 5 mM $MgCl_2$, glycerol, NP40, and protease inhibitors. The samples were homogenized using Ultra-Turrax for 20 s. After centrifugation (14 000 rpm, 15 min, 4°C) the supernatant was clarified from the pellet and its protein content was determined (Pierce, Rockford, IL, USA). For detecting protein levels of phosphorylated protein kinase B (pAkt), Akt and insulin receptor (IR) equal amounts of protein (25 μg) were solubilized in 5 x Laemmli sample buffer. Proteins were separated by SDS-PAGE, transferred to Immobilon-P membranes, blocked, incubated with polyclonal anti-IR (Santa Cruz, CA), anti-pAkt, -Akt and -IR (Cell Signalling, Beverley, MA) primary antibodies (1:1000) and detected by enhanced chemiluminescence after the incubation with HRP-linked secondary antibodies (1:5000). The protein bands were quantified using ImageGauge software (version 3.12, Fuji Photo Film, Tokyo, Japan).

Liver lipid analysis using high performance thin layer chromatography

For analysis of lipid content, livers were homogenized in PBS. Lipids were extracted with Bligh and Dyer's method as described.[17] Lipids were separated by high performance thin layer chromatography (HPTLC) on silica-gel-60 pre-coated plates (Alltech) as described.[18] The amount of lipid (free cholesterol, TG and cholesterylesters) was determined with TINA software (Raytest Isotopen meßgeräte GmbH, Straubenhardt, Germany).

Determination of fibrinogen and serum amyloid-A

Plasma fibrinogen and serum amyloid-A (SAA) levels were determined after 6 weeks on the high fat diet by ELISA as previously described.[19]

Statistical analysis

Results are presented as means ± SD for the number of animals indicated. Differences between experimental groups were determined by the Mann-Whitney U test. The level of statistical significance of the differences was set at $P < 0.05$. Analyses were performed using SPSS 12.0.1 for Windows software (SPSS, Chicago).

Results

Plasma lipid parameters and basal energy metabolism

We observed no differences in body weight between the IL-$10^{-/-}$ and wt mice before and after 6 weeks on a high fat diet. Blood samples taken after 4 h fasting showed no differences between the two groups in plasma TG and cholesterol levels before and after 6 weeks on a high fat diet (Figure 1). To study basal energy metabolism, IL-$10^{-/-}$ mice and wt controls were studied in the metabolic cages after 6 weeks on the high fat diet. Figure 2 shows metabolic characteristics during both the active (night) and inactive (day) periods. We observed no differences in O_2 consumption (3394 ± 636 vs 3201 ± 635 mL/kg/h at night), heat production (0.45 ± 0.09 vs 0.44 ± 0.09 kcal/h at night) or respiratory exchange ratio (RER; 0.83 ± 0.05 vs 0.82 ± 0.05 at night) after 6 weeks on the high fat diet.

Hyperinsulinemic euglycemic clamp studies

We performed hyperinsulinemic euglycemic clamp studies in IL-$10^{-/-}$ mice and wt controls after an overnight fast after 6 weeks on a high fat diet. After overnight fasting no difference in body weight was observed (Table 1). The plasma values of glucose, insulin and FA before and during hyperinsulinemia are shown in Table 1. During hyperinsulinemia glucose levels were maintained at approximately 7 mM and plasma insulin levels were ~ 5 to 10-fold higher when compared to basal conditions. Strikingly, during the hyperinsulinemic period plasma insulin concentrations were ~ 55% lower in IL-$10^{-/-}$ mice compared to wt control mice (1.8 ± 0.8 vs 4.1 ± 1.5 ng/mL;

P < 0.05) despite the infusion of identical amounts of insulin. Basal hepatic glucose production, which equals whole body glucose uptake in the basal state, was not different between IL-10$^{-/-}$ mice and wt controls (47.3 ± 7.0 vs 50.1 ± 4.0 µmol·min^{-1}·kg^{-1}; Figure 3A and B). During hyperinsulinemia whole-body glucose disposal (WGD) increased to a similar level in the two groups (74.6 ± 17.1 *vs* 83.8 ± 25.9 µmol·min^{-1}·kg^{-1}; Figure 3A). No differences in hepatic glucose production (HGP) were observed during hyperinsulinemia (26.5 ± 9.6 vs 26.5 ± 12.0 µmol·min^{-1}·kg^{-1}; Figure 3B). However, after correction for the 55% lower hyperinsulinemic plasma insulin levels, IL-10$^{-/-}$ mice showed a larger increase in insulin-stimulated whole-body glucose uptake. The corrected insulin-mediated decrease (CID) in hepatic glucose production was significantly larger in IL-10$^{-/-}$ mice compared to wt control mice (14.5 ± 6.5 vs 5.0 ± 2.6 µmol·min^{-1}·kg^{-1}/ng plasma insulin; *P* < 0.05; Figure 3C).

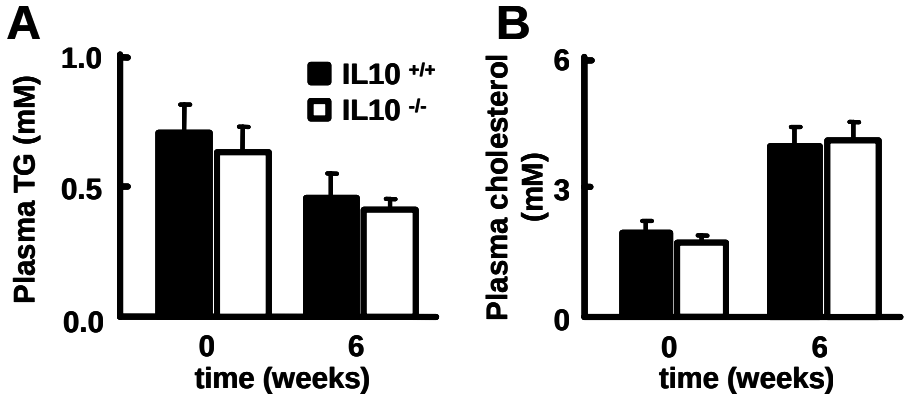

Figure 1. IL-10 deficiency does not affect plasma lipid levels. Plasma lipid levels were measured after 4 h fasting. **A**. Plasma triglycerides (TG). **B**. Plasma cholesterol. (n=12 mice/group)

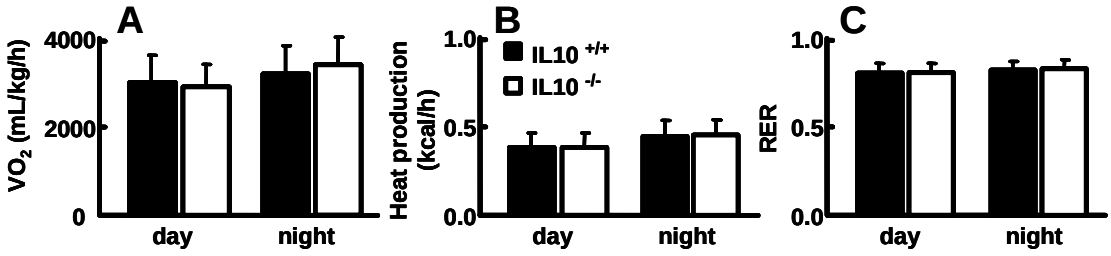

Figure 2. IL-10 deficiency does not affect basal energy metabolism. A. VO2 of IL-10$^{-/-}$ mice and wt controls after 6 weeks on the high fat diet. (n=4) **B**. Heat production of IL-10$^{-/-}$ mice and wt controls after 6 weeks on the high fat diet. (n=4) **C**. RER of the IL-10$^{-/-}$ mice and wt controls after 6 weeks on the high fat diet as measured by indirect calorimetry. (n=4 mice/group)

Table 1. Plasma parameters and glucose infusion rate during the clamp.

	BW	Glucose (mM)		Insulin (ng/mL)		FA (mM)		GIR
		basal	hyper	basal	hyper	basal	hyper	(µmol·min^{-1}·kg^{-1})
IL-10$^{+/+}$	**26.0**	**6.1**	**7.7**	**0.36**	**4.05**	**0.65**	**0.29**	**57.3**
	1.5	*0.5*	*0.8*	*0.13*	*1.50*	*0.09*	*0.05*	*17.9*
IL-10$^{-/-}$	**27.2**	**5.8**	**7.0**	**0.36**	**1.83***	**1.14***	**0.52***	**45.7**
	1.8	*0.5*	*0.9*	*0.04*	*0.63*	*0.14*	*0.31*	*10.8*

The clamp procedure was performed on IL-10$^{-/-}$ mice and wt controls after overnight fasting. Hyperinsulinemia and euglycemia were indeed established during the hyperinsulinemic period (hyper). Plasma insulin levels were ~ 55% lower in the IL-10$^{-/-}$ mice while infusing an identical amount of insulin. Plasma FA were significantly increased in the IL-10$^{-/-}$ mice during the clamp. Under hyperinsulinemia the plasma FA were decreased ~ 40% in both groups, but remained elevated in the IL-10$^{-/-}$ mice. The glucose infusion rate was not different between the groups. (*P < 0.05; n=6-7 mice/group) BW = body weight, GIR = Glucose infusion rate.

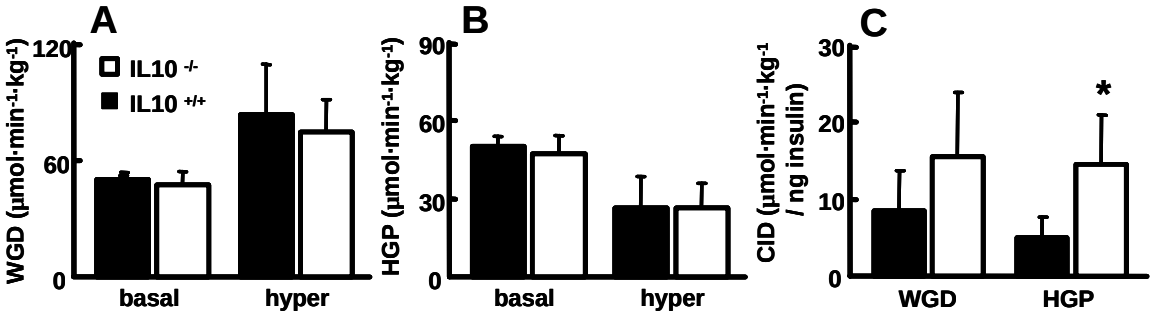

Figure 3. IL-10 deficiency does not affect peripheral or hepatic insulin sensitivity as measured during a hyperinsulinemic euglycemic clamp. Whole-body glucose disposal (WGD; panel **A**.) and hepatic glucose production (HGP; panel **B**.) were measured during the basal period and under hyperinsulinemic conditions using the hyperinsulinemic euglycemic clamp method in both groups. **C**. The insulin-mediated stimulation of whole-body glucose disposal and the inhibition of hepatic glucose production were corrected for the plasma insulin levels (CID) because in the IL-10$^{-/-}$ mice the plasma insulin levels were ~ 55% lower compared to wt mice. (* P < 0.05; n= 6-7 mice/group)

Basal plasma FA levels were significantly increased in the IL-10$^{-/-}$ mice compared to the wt mice after overnight fasting (1.14 ± 0.14 vs 0.65 ± 0.09 mM; $P < 0.05$; Table 1). During hyperinsulinemia plasma FA levels decreased in both groups by about 40% as compared to the respective levels under basal conditions. Nonetheless, plasma FA levels remained significantly higher in IL-10$^{-/-}$ mice (0.52 ± 0.31 *vs* 0.29 ± 0.05 mM; $P < 0.05$).

Hepatic pAkt protein expression levels

To investigate the effect of the hyperinsulinemic euglycemic clamp conditions on insulin signalling in the liver, we measured phosphorylation and protein expression of Akt and IR protein expression. We performed immunoblotting on liver samples from mice, which had undergone the euglycemic hyperinsulinemic clamp. Despite decreased plasma insulin levels, we found increased phosphorylation of Akt in IL-10$^{-/-}$ mice upon insulin stimulation during the clamp compared to wt mice (Figure 4, 12.5 ± 1.4 vs 9.3 ± 2.0 arbitrary units (AU); $P < 0.05$), while Akt and IR protein levels were not changed (2.7 ± 0.3 vs 2.2 ± 0.1 and 8.4 ± 0.5 vs 7.8 ± 1.4 AU, respectively).

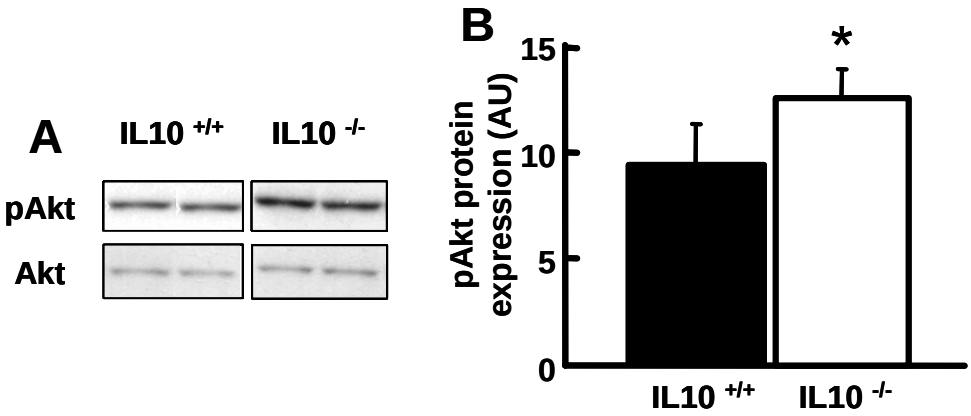

Figure 4. Increased hepatic Akt phosphorylation in IL-10$^{-/-}$ mice during euglycemic hyperinsulinemic clamp conditions. pAkt protein levels were determined using western blotting. Equal amounts of protein (25 µg) for pAkt and Akt expression were loaded, quantified and corrected for loading differences. **A.** Western blot. **B.** Quantification of pAkt protein levels corrected for loading differences (* $P < 0.05$; n=4).

Liver lipid content

Hepatic TG content is inversely related to hepatic insulin sensitivity in some mouse models.[20] In IL-10$^{-/-}$ mice we observed a ~ 54% increase in hepatic TG content

compared to wt mice (168.7 ± 42.3 vs 109.4 ± 42.3 µg TG/mg protein; $P < 0.05$; Figure 5), even though IL-10-deficiency does not alter plasma TG levels (see Figure 1). Liver free cholesterol (FC) content was also increased in IL-10$^{-/-}$ mice (17.7 ± 5.2 vs 11.9 ± 2.7 µg FC/mg protein; $P < 0.05$), whereas the amount of cholesterylesters (CE) was decreased (3.8 ± 1.8 vs 6.3 ± 2.8 µg CE/mg protein; $P = 0.063$). Consequently, the FC/CE ratio was significantly larger in the IL-10 $^{-/-}$ mice (5.9 ± 3.3 vs 2.3 ± 1.4; $P < 0.05$).

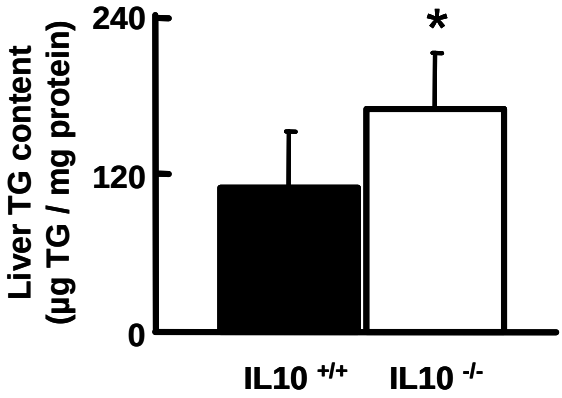

Figure 5. IL-10 protects against hepatic steatosis. Hepatic triglyceride content was determined using high performance thin layer chromatography. (* $P < 0.05$; n= 6-7 mice/group)

Adipose tissue mass

Fatty liver and increased plasma FA are associated with increased visceral adipose tissue mass.[21] Therefore we measured subcutaneous and visceral adipose tissue mass in the IL-10$^{-/-}$ and wt mice and related it to the body weight of the mice. We found that visceral adipose tissue mass was significantly increased in IL-10$^{-/-}$ compared to wt mice (2.0 ± 0.5 vs 1.5 ± 0.7 % of total body weight; $P < 0.05$; Figure 6). The subcutaneous adipose tissue mass was not changed between IL-10$^{-/-}$ and wt mice (0.8 ± 0.2 vs 0.7 ± 0.2 % of total body weight).

Plasma fibrinogen and SAA

To exclude differences in systemic or hepatic inflammation we measured plasma fibrinogen and SAA levels after 6 weeks on the high fat diet in both mouse groups. No differences in fibrinogen (2.9 ± 1.1 vs 2.6 ± 0.8 mg/mL) or SAA levels (182 ± 170 vs 217 ± 291 µg/mL) were observed.

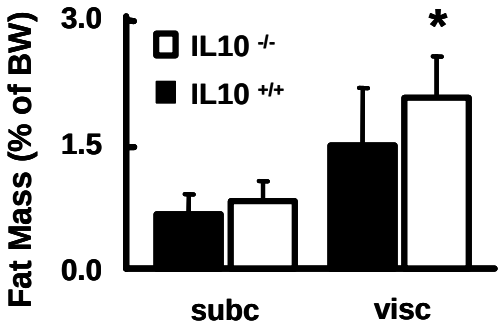

Figure 6. Increased visceral adipose tissue mass in IL-10$^{-/-}$ mice. Visceral and subcutaneous adipose tissue was quantified as a percentage of total body weight. (* P < 0.05; n= 6-7 mice/group)

Discussion

Our study is the first to establish the direct consequences of IL-10 deficiency on hepatic and peripheral insulin sensitivity. Our data show, that basal IL-10 production protects against hepatic steatosis during high fat feeding. However, endogenous IL-10 secretion does not improve hepatic or whole-body insulin sensitivity during high fat feeding as assessed by the hyperinsulinemic euglycemic clamp technique. These observations argue against a simple protective role of endogenous IL-10 secretion in insulin resistant states, at least within our mouse model. Nonetheless, our data also indicate that endogenous IL-10 secretion is not metabolically inert, since we documented clear effects of IL-10 deficiency on hepatic and peripheral lipid metabolism.

We observed no differences in the plasma levels of TG and total cholesterol between high fat-fed IL-10$^{-/-}$ and wt mice. This is in concordance with a previous study [22] in IL-10$^{-/-}$ mice on an apolipoprotein E-deficient background. In those mice a shift of cholesterol from VLDL to LDL was observed, although total cholesterol levels remained unchanged. Conversely, over-expression of IL-10 in mice on a LDLr$^{-/-}$ background led to a significant decrease in total cholesterol.[23] In that study a high correlation between plasma total cholesterol levels and plasma IL-10 concentration was found. In accordance, several studies in humans documented an inverse association between plasma IL-10 and lipid levels.[11,24] In contrast, this association does not hold in the complete absence of IL-10, as we show in our study in IL-10$^{-/-}$ mice on a Black6 background and is shown by others in apolipoprotein E knockout

mice.[11,22] We can not exclude the possibility that, in the absence of any IL-10 production capacity, compensatory mechanisms prevent dysregulation of the lipid metabolism.

We expected mice lacking IL-10 to be more catabolic in comparison to wild type mice, since they lack this anti-inflammatory cytokine. Interestingly, when we compared basal metabolic characteristics we found absolutely no differences in heat production, food intake, VO_2, VCO_2 and respiratory exchange ratio. Apparently, under basal conditions IL-10 is not a crucial cytokine in energy metabolism. LPS-mediated activation of the immune system may elucidate a more important role for IL-10. However, that would be a model of infection rather than a model of metabolic regulation *per se*.

Strikingly, we found decreased hyperinsulinemic plasma insulin concentrations in the IL-$10^{-/-}$ mice compared to the wild type controls although we infused identical amounts of exogenous insulin. The amount of insulin infused in our study protocols normally results in plasma insulin levels of ~ 4-6 ng/mL as were observed our wild type control mice.[25,26] Thus, the absence of any difference in hepatic glucose production and peripheral glucose uptake between IL-$10^{-/-}$ mice and wt controls during the clamp experiment occurred despite lower plasma insulin levels in the IL-$10^{-/-}$ mice. This combination of data suggests improved insulin sensitivity in IL-$10^{-/-}$ mice, rather than the initially hypothesized decreased insulin sensitivity. In addition, the data indicate that IL-10 deficiency is associated with a higher rate of plasma clearance of insulin, for reasons presently unknown.

We subsequently evaluated the activity of important markers of the hepatic insulin signalling cascade in livers obtained from hyperinsulinemic IL-$10^{-/-}$ mice and wt controls. We found, that phosphorylation of Akt was significantly increased in IL-$10^{-/-}$ mice despite lower plasma insulin concentrations under hyperinsulinemia, although Akt and insulin receptor expression were not changed. Therefore, both the *in vivo* glucose kinetic data obtained during hyperinsulinemia, as well as these markers of the insulin signalling cascade point to increased hepatic insulin sensitivity, rather than the expected hepatic insulin resistance in IL-$10^{-/-}$ mice.

IL-10 deficiency is associated with major changes in hepatic lipid content, reflected in increased TG content upon high fat feeding. In many mouse models and in humans, positive correlations exist between hepatic steatosis and hepatic insulin

resistance.[20,27-30] However, there are also many examples of steatosis, that are not associated with hepatic insulin resistance, including the treatment of mice with thiazolidinediones or LXR agonists or the inhibition of fatty acid oxidation.[31-33] Obviously, the relation between steatosis and hepatic insulin resistance is not straightforward, because other factors with complex interactions may be involved. The increased liver TG content may be due to increased plasma FA flux into the liver after overnight fast. Plasma FA levels were significantly increased in the IL-10$^{-/-}$ mice both in the basal state and under hyperinsulinemia (Table 1). This may result from increased lipolysis and release of FA from the increased visceral adipose tissue store in the IL-10$^{-/-}$ mice compared to control mice. In both groups of mice plasma FA as a measure of adipose tissue lipolysis is deceased by ~ 40% under hyperinsulinemia, suggesting no change in adipose tissue insulin sensitivity. However, in the IL-10$^{-/-}$ mice the plasma FA level remains significantly increased compared to controls. Increased visceral adipose tissue mass is associated with increased plasma FA and fatty liver in humans.[21] A potential explanation for this association may be the portal delivery of FA to the liver.[34] Subsequently, upon uptake by the liver these FA may be esterified into TG that may accumulate within the liver, since hepatic VLDL-TG production is not increased in IL-10$^{-/-}$ mice. [23] Alternatively, we cannot exclude the involvement of other changes in intra-hepatic fatty acid metabolism like an increase in the expression of lipogenic enzymes, or a decrease in fatty acid oxidation. Although the increase in hepatic cholesterol content could be due to increased cholesterol synthesis in the liver, the increased FC/CE ratio indicates an impairment of the esterification of cholesterol into cholesteryl esters. The mechanism behind this observation is beyond the scope of this paper.

We measured plasma fibrinogen and SAA in the IL-10$^{-/-}$ and the wt control mice. Although fibrinogen and SAA levels increased in both groups in time on the high fat diet, no difference in the plasma levels of these markers of systemic and hepatic inflammation were observed between the two genotypes. Therefore, we conclude that the effects in IL-10 deficient mice do not simply reflect a higher state of chronic (hepatic) inflammation.

In summary, IL-10 deficiency alters peripheral and hepatic lipid metabolism. However, this study does not support a causal role of IL-10 in the protection against diet-induced hepatic insulin resistance and other metabolic disturbances.

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