Scavenger Receptor Class B Type I Mediates the Selective Uptake of High-Density Lipoprotein–Associated Cholesteryl Ester by the Liver in Mice

May Brundert, Anne Ewert, Joerg Heeren, Barbara Behrendt, Rajasekhar Ramakrishnan, Heiner Greten, Martin Merkel, Franz Rinninger

Objective—High-density lipoprotein (HDL) cholesteryl esters (CE) are taken up by liver and adrenals selectively, ie, independent from particle internalization. Class B type I scavenger receptor (SR-BI) mediates this uptake in vitro. The role of SR-BI in HDL metabolism was explored in mice.

Methods and Results—Mice with a mutation in the SR-BI gene (SR-BI KO) and wild-type (WT) littermates were used. Mutants had increased HDL cholesterol. HDL was labeled with ^{125}I (protein) and [^{3}H] (CE). After HDL injection, blood samples were drawn and finally the mice were euthanized. In WT, the plasma decay of HDL-associated [^{3}H] is faster compared with ^{125}I and this represents whole-body selective CE uptake. In SR-BI KO, the decay of both tracers is similar, yielding no selective CE removal. In WT liver and adrenals, uptake of [^{3}H] is higher than ^{125}I, showing selective uptake. In SR-BI KO, liver uptake of [^{3}H] and ^{125}I are similar, proposing no selective HDL CE uptake. In SR-BI KO adrenals, selective uptake is reduced; however, even in the absence of SR-BI, this uptake is detected using WT-HDL.

Conclusions—SR-BI mediates selective uptake of HDL CE by the liver. In adrenals, an alternative mechanism or mechanisms can play a role in selective CE uptake. (***Arterioscler Thromb Vasc Biol*. 2005;25:143-148.)**

Key Words: HDL ■ SR-BI ■ cholesterol ■ liver ■ receptor

High-density lipoprotein (HDL) plays a critical role in cholesterol homeostasis.[1] HDL presumably removes cholesterol from peripheral tissues.[1,2] After esterification in plasma, HDL-associated cholesteryl esters (CE) are delivered to other lipoprotein fractions or to tissues.[1,2] One mechanism that mediates the delivery of HDL-associated CE to organs is the selective lipid uptake pathway.[3] In this process, HDL CE are internalized by cells independently from the uptake of the HDL particle. This selective lipid uptake appears to be important for the transport of cholesterol to steroidogenic tissues for hormone synthesis and to the liver.[3] In this central organ of lipid metabolism, HDL cholesterol is secreted into bile, used for bile acid synthesis, or packaged and secreted in newly synthesized lipoproteins.[2] This HDL-mediated transport of cholesterol from extrahepatic tissues to the liver is designated reverse cholesterol transport.[1]

The class B type I scavenger receptor (SR-BI) is a cell surface HDL receptor that binds HDL.[4] In cultured cells, SR-BI mediates the selective HDL CE uptake. In rodents, SR-BI is most abundantly expressed in liver and steroidogenic tissues,[4] which are those tissues most actively engaged in selective lipid uptake in vivo.[3,5] In mice, hepatic overexpression of SR-BI reduces plasma HDL and increases biliary cholesterol.[6] In contrast, rodents with a targeted null mutation in the SR-BI gene have an increase in plasma HDL.[7] Taken together, evidence has been presented that SR-BI may be a physiologically relevant HDL receptor in vivo.

The role of SR-BI in HDL metabolism was explored in mice with an attenuated expression of this receptor in liver and adrenals.[8] Studies using radiolabeled HDL showed that a 53% decrease in hepatic SR-BI expression is associated with a 47% reduction in HDL selective CE uptake by the liver. This observation is consistent with an important function of SR-BI in HDL metabolism in vivo. However, this study[8] did not address the issue of whether SR-BI is the only molecule that mediates HDL selective CE uptake or whether a pathway or pathways distinct from this receptor contribute to hepatic CE uptake in vivo. With respect to the mechanism of selective uptake, it was proposed that lipolytic enzymes like hepatic lipase[9,10] and lipoprotein lipase[11] may mediate HDL selective CE uptake. Besides, protein-independent lipid–lipid interactions could play a role in the selective CE transfer.[12]

In this study, the role of SR-BI in HDL metabolism in vivo was explored. Mice with a targeted homozygous null mutation in the gene encoding SR-BI (SR-BI KO) and their wild-type (WT) littermates were used as model.[7] Murine

Original received January 28, 2004; final version accepted September 30, 2004.

From University Hospital Hamburg-Eppendorf (M.B., A.E., J.H., B.B., H.G., M.M., F.R.), Hamburg, Germany; and the College of Physicians and Surgeons of Columbia University (R.R.), New York, NY.

Correspondence to Dr Franz Rinninger, University Hospital Hamburg-Eppendorf, Department of Medicine, Martinistrasse 52, 20246 Hamburg, Germany. E-mail Rinninger@uke.uni-hamburg.de

Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000149381.16166.c6**

HDL was radiolabeled in the protein (^{125}I), as well as in the lipid ([^{3}H]) moieties.[13] In WT mice, HDL CE are selectively taken up by liver and adrenals.[3] In contrast, the livers of SR-BI–deficient mutants displayed no selective CE uptake from HDL. In adrenals without SR-BI expression, radiolabeled HDL from SR-BI KO mice yielded no selective CE uptake, whereas substantial selective lipid uptake was detected when radiolabeled HDL originated from WT rodents. HDL metabolism by hepatocytes isolated either from WT or from SR-BI KO mice was investigated. These in vitro studies qualitatively agree with experiments performed in vivo. In summary, this investigation shows that SR-BI is a major receptor that mediates HDL selective CE uptake by the liver in mice. However, in adrenals, an alternative mechanism or mechanisms can contribute to this lipid uptake.

Methods

For an expanded Methods section, please see http://atvb.ahajournals.org.http://atvb.ahajournals.org.

Mice

Mice with a homozygous null mutation in the SR-BI gene (SR-BI KO) and the respective littermates were used.[7]

Lipoprotein Preparation

HDL was isolated by ultracentrifugation from WT (WT-HDL) and from SR-BI KO (homozygous) mouse plasma (SR-BI KO-HDL).[8] HDL was radiolabeled with ^{125}I-tyramine cellobiose (^{125}I-TC) and [^{3}H]cholesteryl oleyl ether ([^{3}H]CEt).[13]

HDL Metabolism in Mice

Radiolabeled HDL was injected in the tail veins and blood samples were drawn periodically for 24 hours after injection.[3,5] Plasma samples were analyzed for ^{125}I-TC and for [^{3}H]CEt.[5] Finally, the animals were anesthetized and euthanized, the organs were harvested, and tissue content of ^{125}I-TC and of [^{3}H]CEt was analyzed.[5]

Mouse Hepatocyte Isolation

Hepatocytes were prepared from livers by collagenase perfusion.[14]

Uptake Assay for Radiolabeled HDL

Hepatocytes were incubated (37°C, 2 hours) in DMEM containing radiolabeled HDL.[15] Finally, uptake of HDL tracers was analyzed.

Miscellaneous

Cholesterol, phospholipids, and triglycerides were measured with enzymatic assays. Plasma lipoproteins were fractionated by fast protein liquid chromatography (FPLC).[8]

Statistics and Calculations

Values are means±SEM. Significance of differences was calculated using Student *t* test.

The decay of HDL-associated ^{125}I-TC and [^{3}H]CEt yielded plasma fractional catabolic rates (plasma-FCRs).[16] To analyze the activities of tissues in HDL uptake and to compare the uptake of the protein and CE component by tissues, organ-FCRs for both HDL tracers were determined.[3]

For hepatocytes, uptake of ^{125}I-TC/[^{3}H]CEt-HDL is shown in terms of apparent HDL particle uptake.[3,15]

Results

Mice

A targeted mutation in the gene encoding SR-BI induced an increase in plasma cholesterol. In male WT mice, fasting plasma cholesterol was 101.2±2.9 mg/dL (n=27 mice), and in SR-BI KO (homozygous) animals the respective value was 189.7±5.7 (n=27 mice, $P<0.0001$). This corresponds to an increment of 87%. To analyze which lipoprotein fraction increased because of the SR-BI deficiency, fast protein liquid chromatography (FPLC) analysis of plasma cholesterol was performed (data not shown).[8] Compared with WT, the increase in plasma cholesterol in SR-BI KO mice was predominantly caused by an increase in HDL cholesterol. However, the HDL peak from SR-BI KO mice was shifted somewhat to the left, suggesting a small increase in HDL particle size.[7] Besides, HDL was isolated from plasma of WT and of SR-BI KO (homozygous) mice by ultracentrifugation.[15] In WT rodents, HDL cholesterol was 44.6±3.5 mg/dL (n=3 mice); in SR-BI KO mice, HDL cholesterol was 87.5±0.5 mg/dL (n=3 mice, $P=0.0003$). This corresponds to an increase of 96%. The composition of WT HDL and of SR-BI KO-HDL was explored (Table 1). SR-BI KO-HDL is enriched in cholesterol compared with WT-HDL, and this increase is caused by an increment in unesterified cholesterol.[17] In summary, an SR-BI deficiency in mice increased plasma HDL cholesterol and yielded somewhat larger lipoprotein particles, which are enriched in cholesterol.

TABLE 1. Chemical Compositions of Murine HDL

	WT-HDL	SR-BI KO-HDL
	% of Total Mass	
Unlabeled HDL		
Total cholesterol	12.3±1.3*	19.5±1.5*
Unesterified cholesterol	3.2±0.2†	11.1±0.8†
Phospholipid	21.7±2.2	22.8±2.5
Triglyceride	1.5±0.5	1.2±0.5
Protein	64.4±2.9	56.5±3.9
Unesterified:total cholesterol	0.26	0.56
^{125}I-TC-/ [^{3}H]CEt-HDL		
Total cholesterol	12.6±0.4‡	17.9±0.9‡
Unesterified cholesterol	3.4±0.1§	10.1±0.7§
Phospholipid	24.1±0.6	25.0±1.1
Triglyceride	0.5±0.04	0.4±0.05
Protein	62.8±0.9	56.7±1.9
Unesterified:total cholesterol	0.26	0.56

Blood was harvested in parallel from fasted WT or from SR-BI KO (homozygous) mice. Subsequently, plasma HDL (d=1.063 to 1.21 g/ml) was isolated by ultracentrifugation. Thereafter in some cases, these preparations were radiolabeled as described in Methods. Finally, total cholesterol, unesterified cholesterol, phospholipid, triglyceride, and protein were analyzed. Values for unlabeled HDL are means±SEM of n=3 independent preparations, data for labeled HDL are means±SEM of n=7 preparations. Within each sample, analysis was done at least in duplicate.

*P=0.02; †P=0.0008; ‡P=0.0003; §$P<0.0001$.

HDL Metabolism of Mice

HDL was isolated from WT or from SR-BI KO (homozygous) mice and thereafter radiolabeled in the protein (^{125}I-tyramine cellobiose) and in the CE ([^{3}H]cholesteryl oleyl ether) moieties.[13]

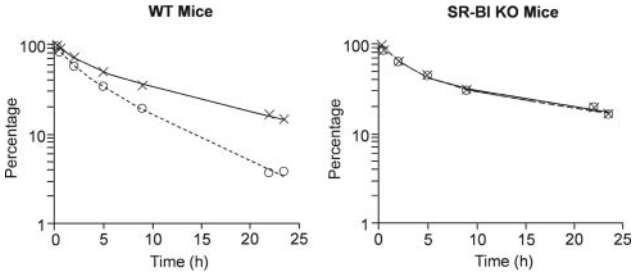

Figure 1. Plasma decay kinetics of ^{125}I-TC/[^{3}H]CEt WT HDL in WT mice or in SR-BI KO mice. ^{125}I-TC/[^{3}H]CEt WT HDL was injected in a WT or in an SR-BI KO (homozygous) mouse. Thereafter, during a 24-hour interval, periodic blood samples were harvested and plasma was analyzed for ^{125}I-TC (crosses) and [^{3}H]CEt (circles). The y-axis represents the fraction of the tracer in plasma (%). Shown is a typical experiment; n=10 WT and n=10 SR-BI KO mice.

^{125}I-TC/[^{3}H]CEt WT HDL was injected in WT or in SR-BI KO (homozygous) mice (Figure 1). Thereafter during a 24-hour interval, periodic blood samples were harvested for analysis of ^{125}I-TC and [^{3}H]CEt. In WT, the plasma decay of HDL-associated [^{3}H]CEt is faster compared with ^{125}I-TC. This experiment shows selective HDL CE clearance from plasma in controls. In contrast, in SR-BI KO, the decay of both HDL tracers is similar (Figure 1). This experiment yields no selective HDL CE clearance in SR-BI KO rodents.

From plasma decay curves (Figure 1), fractional catabolic rates (plasma-FCRs) were calculated (Table 2).[16] In WT, the plasma-FCR for HDL-associated [^{3}H]CEt is higher compared with ^{125}I-TC. This differential decay shows selective CE uptake by tissues from HDL in WT mice.[3,18] In SR-BI KO animals, the plasma FCRs for both HDL tracers are similar. This analysis yields no selective CE uptake from plasma HDL by the whole body in SR-BI–deficient mice.

To investigate tissue sites of HDL uptake, the mice were euthanized 24 hours after ^{125}I-TC-/[^{3}H]CEt WT HDL injection.[3] Thereafter, tissues were harvested for analysis of the respective tracer content. Based on plasma-FCRs and on fractional tracer recovery of tissues, organ-FCRs for each HDL tracer were calculated.[3] These organ-FCRs represent the fraction of the plasma pool of the traced HDL component cleared per hour by a tissue.

For the liver of WT mice, the organ-FCR for HDL-associated [^{3}H]CEt is higher compared with ^{125}I-TC (Figure 2A). The difference in organ-FCRs between both tracers ([^{3}H]CEt–^{125}I-TC) represents selective CE uptake from HDL by the liver,[3] and this rate is substantial in WT mice. In contrast, in SR-BI KO mice, the hepatic organ-FCR for [^{3}H]CEt is significantly reduced compared with WT (Figure 2A). The organ-FCRs for [^{3}H]CEt and ^{125}I-TC for the liver were similar in SR-BI KO mice, and the difference between these rates yielded only a minor selective CE uptake. Thus, hepatic HDL selective CE uptake is reduced by 91% in the absence of SR-BI.

^{125}I-TC/[^{3}]CEt WT HDL uptake by adrenals is presented in Figure 2B. In WT mice, organ-FCRs for [^{3}H]CEt are higher compared with ^{125}I-TC; the difference between both tracers yields selective CE uptake, and this rate was substantial in normal glands. In adrenals of SR-BI KO mice, the organ-FCR for [^{3}H]CEt decreased, whereas the respective rate for ^{125}I-TC increased (compared with WT). Thus, selective HDL CE uptake ([^{3}H]CEt–^{125}I-TC) by adrenals decreased to some extent in glands from SR-BI KO mice (Figure 2B). However, even in the absence of SR-BI, substantial selective CE uptake could be detected in receptor-deficient glands. Thus, in this steroidogenic tissue, SR-BI mediates a fraction of HDL selective CE uptake in vivo, whereas significant selective CE uptake occurs independent from this receptor. In adrenals of SR-BI KO mice, the organ-FCR for HDL-associated ^{125}I-TC is higher compared with the respective rate in WT (Figure 2B). HDL protein-associated ^{125}I-TC traces HDL holo-

TABLE 2. Plasma Decay of ^{125}I-TC-/[^{3}H]CEt-WT-HDL in WT or in SR-BI KO Mice and Tracer Uptake Rates by Tissues

	WT Mice			SR-BI KO Mice		
	Cholesterol	^{125}I	[^{3}H]	Cholesterol	^{125}I	[^{3}H]
Plasma cholesterol, mg/dl	105.8±4.8			188.2±9.5		
Plasma-FCRs, pools/h		0.0713±0.0052	0.1364±0.0078†		0.0853±0.0064	0.0780±0.0079†
Organ-FCRs, $\times h^{-1}\times 10^{3}$						
Kidneys		2.54±0.22	0.683±0.048*		3.12±0.28	0.482±0.050*
Spleen		0.750±0.069†	2.24±0.21*		1.34±0.09†	1.70±0.11*
Stomach		0.638±0.080*	0.607±0.063*		1.61±0.30*	1.47±0.34*
Intestine		5.39±0.85*	3.18±0.39		8.48±1.0*	4.19±0.70
Brain		0.0181±0.001	0.0324±0.003†		0.0219±0.003	0.0166±0.003†
Heart		0.375±0.032	0.351±0.027		0.475±0.041	0.300±0.037
Lungs		0.490±0.046	0.967±0.089†		0.482±0.062	0.397±0.047†
Carcass		31.9±1.5	38.3±1.8		36.6±3.7	30.8±3.2

^{125}I-TC-/[^{3}H]CEt-WT-HDL was injected in WT or in SR-BI KO (homozygous) mice. During the subsequent 24-hour interval, blood was harvested periodically to determine the plasma decay of both tracers. Twenty-four hours after tracer injection, the animals were humanely killed and tissues were analyzed for both tracers. Plasma-FCR's and organ-FCR's for ^{125}I-TC (^{125}I) and [^{3}H]CEt ([^{3}H]) were calculated as described in Methods. Values are means±SEM of n=10 mice in each group.

*P<0.05; †P<0.005.

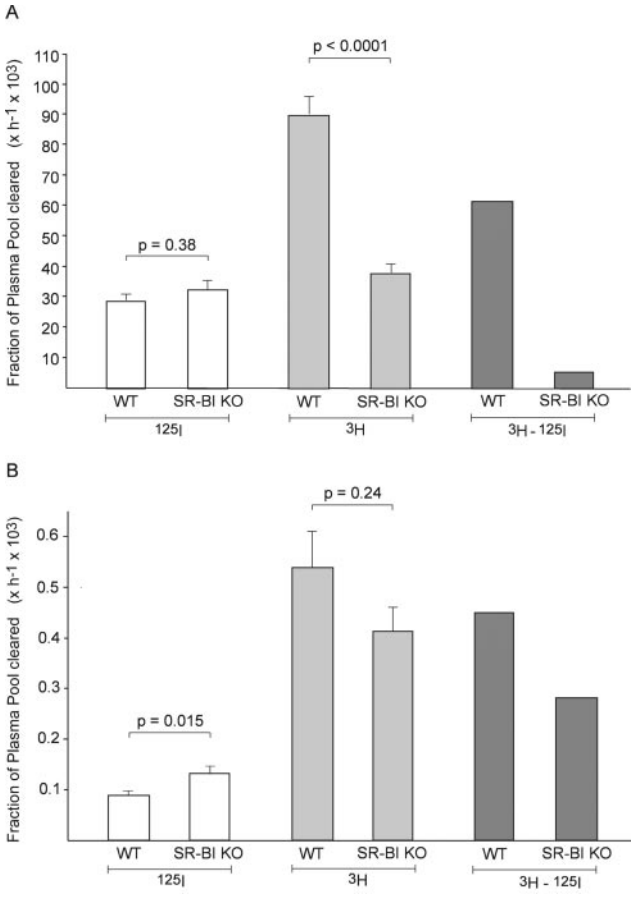

Figure 2. Uptake of ^{125}I-TC/[^{3}H]CEt WT HDL by liver and adrenals of WT or of SR-BI KO mice. ^{125}I-TC/[^{3}H]CEt WT HDL was injected in WT and in SR-BI KO (homozygous) mice. During the subsequent 24-hour interval, blood was harvested periodically for analysis of plasma FCRs for both tracers. Twenty-four hours after tracer injection, the animals were euthanized, perfused with saline, and livers (A) and adrenals (B) were analyzed for the content of each tracer. Organ FCRs for ^{125}I-TC (^{125}I) and [^{3}H]CEt ([^{3}H]) were calculated as outlined in Methods. Values are means±SEM of n=10 mice in each group.

particle metabolism.[19] Assuming this, a lack of SR-BI in adrenal glands increases HDL holo-particle uptake.

^{125}TC/[^{3}H]CEt WT HDL uptake by other murine tissues was explored (Table 2). In WT, in kidneys, the organ-FCRs for HDL-associated ^{125}I-TC are higher compared with [^{3}H]CEt. This result is consistent with a role of the kidneys in HDL apolipoprotein catabolism.[20] In WT mice, in spleen, brain, lungs, and carcass, the organ-FCRs for HDL-associated [^{3}H]CEt are higher compared with ^{125}I-TC, and this shows selective uptake (Table 2). In stomach, intestine, and heart of WT, the uptake rates for both HDL tracers are similar, yielding no selective CE uptake.[18]

In SR-BI KO (homozygous) mice, the uptake rates for [^{3}H]CEt by kidneys were reduced; however, also in this case, the uptake rates for ^{125}I-TC were in excess of those caused by the lipid tracer (Table 2). In SR-BI KO animals, in spleen, brain, lungs, and carcass, the organ-FCRs for HDL-associated [^{3}H]CEt decreased compared with WT; the difference in uptake between [^{3}H]CEt and ^{125}I-TC yields no or a diminished HDL-selective CE uptake in these organs in SR-BI–deficient mice. For stomach, intestine, and heart, organ-FCRs for ^{125}I-TC and [^{3}H]CEt yielded no selective CE uptake in SR-BI KO rodents.

A quantitative estimate on HDL uptake by tissues and the effect of an SR-BI deficiency can be obtained from Figure 2 and Table 2. Comparing all organ-FCRs for tissue sites of HDL tracer uptake, the liver in WT mice yielded the highest uptake rate for [^{3}H]CEt and a high rate for ^{125}I-TC. This analysis shows that quantitatively, the liver is the major organ site for HDL CE and for HDL selective CE uptake in normal mice.[18] In SR-BI KO, the dominant quantitative change in HDL internalization by tissues is a decrease in hepatic [^{3}H]CEt uptake (Figure 2A); the organ-FCR for this tracer decreased by 58% in SR-BI KO liver compared with WT. Carcass had relatively high uptake rates for HDL tracers in WT and in SR-BI KO mice. It is important to note that carcass represented 75.9±0.6% (n=11 mice) of the total body weight of the mice. Compared with liver and carcass, the remaining organ-FCRs are quantitatively small (Table 2).

In the experiments presented, in both groups of mice, HDL metabolism was examined using WT-HDL, ie, the same tracer was used in animals with and without SR-BI. However, SR-BI KO-HDL is enriched in cholesterol (Table 1). To investigate whether this difference of the donor particle modifies HDL selective CE uptake in SR-BI KO mice, the metabolism of ^{125}I-TC/[^{3}H]CEt SR-BI KO HDL was explored in SR-BI KO (homozygous) rodents as well; for comparison, the turnover of ^{125}I-TC/[^{3}H]CEt WT HDL was investigated in WT animals (Table I, available online at http://atvb.ahajournals.org). After injection of ^{125}I-TC/[^{3}H]CEt SR-BI KO HDL in SR-BI KO mice, plasma-FCRs and liver and adrenal organ-FCRs for both tracers were very similar. Therefore, no selective HDL CE clearance from plasma and no selective CE uptake by liver and adrenals could be detected. In WT mice using ^{125}I-TC/[^{3}H]CEt WT HDL, the plasma-FCRs and the liver and adrenal organ-FCRs for [^{3}H]CEt were higher compared with ^{125}I-TC. Again, these results yielded selective HDL CE clearance from plasma, liver, and adrenals in WT mice. Uptake of ^{125}I-TC/[^{3}H]CEt SR-BI KO HDL by nonhepatic and nonsteroidogenic tissues of SR-BI KO mice (Table I) was similar compared with the experiments using ^{125}I-TC/[^{3}H]CEt WT HDL in SR-BI KO animals (Table 2).

Taken together, the composition of the HDL tracer has no significant effect on HDL selective CE uptake by the liver. However, the composition of the HDL particle can modify selective CE uptake by adrenals in SR-BI–deficient mice.

HDL Metabolism of Murine Hepatocytes

The function of SR-BI in hepatic HDL metabolism was investigated in vitro in primary hepatocytes.[14] After preparation from either WT or from SR-BI KO mouse liver, these cells were incubated in medium containing ^{125}I-TC/[^{3}H]CEt WT HDL or ^{125}I-TC/[^{3}H]CEt SR-BI KO HDL.[3,15] Finally, cellular tracer content was analyzed and expressed in terms of apparent HDL particle uptake.

Uptake of ^{125}I-TC/[^{3}H]CEt WT HDL by hepatocytes isolated from WT is shown in Figure 3. Apparent HDL particle uptake according to ^{125}I-TC increased as the HDL concentration in the medium increased. Apparent HDL particle uptake

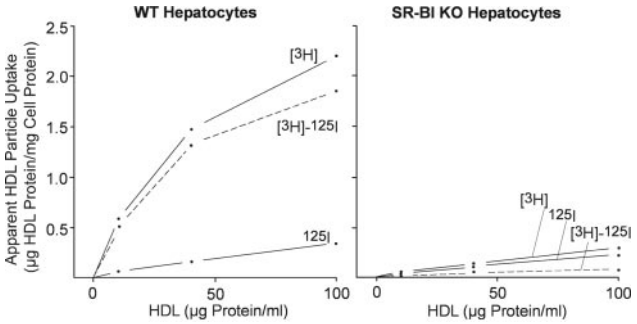

Figure 3. Uptake of ^{125}I-TC/[^{3}H]CEt WT HDL by hepatocytes isolated from WT or from SR-BI KO mice. Hepatocytes were isolated from a WT or from an SR-BI KO (homozygous) mouse. These cells incubated (37°C, 2.0 hours) in medium containing ^{125}I-TC/[^{3}H]CEt WT HDL and the respective concentrations are given in the abscissa. Finally, cells were harvested and apparent HDL particle uptake was analyzed as outlined in Methods. Values are means of n=2 incubations; variation from the mean was <8%. Three independent similar experiments for each type of hepatocyte yielded qualitatively identical results.

caused by [^{3}H]CEt was in excess of that according to ^{125}I-TC, and this lipid uptake increased in a dose-dependent manner as well. The difference in HDL uptake between [^{3}H]CEt and ^{125}I-TC yields selective CE uptake.[19] This CE uptake increased in hepatocytes isolated from WT as a function of the HDL concentration. In parallel, uptake of the identical ^{125}I-TC/[^{3}H]CEt WT HDL by hepatocytes isolated from SR-BI KO mice was investigated (Figure 3). In these SR-BI–deficient cells, uptake of [^{3}H]CEt was reduced throughout the entire concentration range and this yielded a low rate of HDL selective CE uptake ([^{3}H]CEt–^{125}I-TC) by these mutant hepatocytes.

Discussion

A major effect of the loss of SR-BI expression in mice is an increase in plasma cholesterol. This change in phenotype is primarily caused by an increase in HDL cholesterol.[7] HDL particles from SR-BI KO mice are enriched in cholesterol compared with HDL from WT controls,[7] and this increase is caused by an increment in unesterified cholesterol.[17] These modifications in HDL concentration and in HDL composition in the absence of SR-BI are consistent with a function of this protein as an HDL receptor, which mediates HDL selective CE uptake in mice.[4]

The role of SR-BI in HDL metabolism was investigated in mice in the presence or absence of SR-BI. In WT, the plasma decay of the HDL CE tracer is faster compared with the protein tracer. This differential removal indicates whole-body selective CE uptake in normal rodents.[18] In contrast, in SR-BI KO mice, the plasma decay of both HDL tracers is similar, showing a lack of whole-body selective CE clearance from plasma HDL in SR-BI–deficient mice. These observations suggest a key role of SR-BI in plasma HDL CE metabolism in vivo.

With respect to tissue sites of HDL metabolism, in WT mice the liver had the highest organ-FCR for HDL-associated [^{3}H]CEt, suggesting a dominant role of this organ for CE clearance.[3] As expected, substantial HDL selective CE uptake could be detected in liver and adrenals of WT mice.[18] In qualitative agreement with the liver in vivo, primary hepatocytes from WT mice displayed HDL selective CE uptake in vitro. Thus, liver and adrenal glands are physiologically relevant tissues for HDL selective CE uptake in mice with SR-BI expression.[3,18]

HDL uptake by nonhepatic, nonsteroidogenic organs of WT mice revealed for spleen, stomach, brain, heart, and lungs low uptake rates for both HDL tracers compared with the liver.[18] Quantitatively, these organs have a small contribution to whole-body HDL catabolism. Some selective CE uptake was observed in spleen, brain, lungs, and carcass in WT mice. SR-BI is expressed in brain[21] and in macrophages,[22] and this presumably explains selective HDL lipid uptake by the central nervous system and by macrophage-rich organs like spleen and lungs. In kidneys, the uptake of HDL-associated ^{125}I-TC was higher compared with [^{3}H]CEt, and this is because of a preferential renal catabolism of HDL apolipoproteins.[20]

The effect of an SR-BI deficiency on HDL metabolism by tissues was investigated in SR-BI KO mice.[7] Because the composition of SR-BI KO HDL and of WT HDL is different,[17] the metabolism of both preparations was explored. In the liver, an SR-BI deficiency decreased the uptake of HDL-associated [^{3}H]CEt by 58% or 69% (WT HDL or SR-BI KO HDL) compared with the normal organ. In these SR-BI–deficient animals, the hepatic uptake of HDL-associated ^{125}I-TC was essentially unchanged using both HDL preparations. As a result, in the SR-BI–deficient liver, HDL selective CE uptake decreased by 91% or 99% (WT HDL or SR-BI KO HDL). In agreement with these in vivo experiments, expression of SR-BI and the composition of the HDL tracer played a similar role for HDL selective CE uptake by hepatocytes in vitro. In SR-BI–deficient liver cells, radiolabeled WT HDL yielded a very low rate of HDL selective CE uptake, and this internalization was even lower in the presence of SR-BI KO HDL. These in vivo and in vitro experiments provide evidence that SR-BI is the major molecule for HDL selective CE uptake by the liver. This conclusion is true irrespective of the composition of the HDL ligand and is in line with recent studies.[17] However, in the case of cholesterol-poor WT HDL, SR-BI–independent pathway or pathways evidently contribute to a minor degree to hepatic HDL selective CE uptake (Figure 2).

In adrenal glands of SR-BI KO mice, the metabolism of both radiolabeled WT HDL and of SR-BI KO HDL was explored. Uptake of [^{3}H]CEt decreased by 23% or 47% (WT HDL or SR-BI KO HDL) compared with WT. Uptake of ^{125}I-TC significantly increased by 46% or by 80% (WT HDL or SR-BI KO HDL) in receptor-deficient adrenals. HDL selective CE uptake decreased by 42% (WT HDL) and was abolished (SR-BI KO HDL) in adrenal glands of SR-BI KO mice. These results provide evidence for at least 2 mechanisms for HDL selective CE uptake in adrenals. One is dependent on SR-BI, whereas the other is distinct. The SR-BI–dependent mechanism for selective CE uptake is influenced by the composition of the tracer particle. In the case of the cholesterol-enriched SR-BI KO HDL, no selective CE uptake is observed. However, a cholesterol-poor radiolabeled WT HDL yields substantial selective HDL CE uptake

by adrenals from SR-BI–deficient mice, and this internalization must be mediated by SR-BI–independent pathway. Thus, both tissue SR-BI expression and the composition of the HDL particle can determine the rate of HDL selective CE uptake by adrenals. In these glands of mutant mice, the uptake of HDL-associated ^{125}I-TC increased compared with WT. The HDL protein tracer represents the metabolism of HDL holoparticles.[19] Then, the increase in uptake of HDL-associated ^{125}I-TC indicates an increase in HDL particle internalization in SR-BI–deficient adrenals. Adrenal cholesterol is decreased by 72% in SR-BI KO mice.[7] Therefore, it can be speculated that an adrenal cholesterol depletion induces a compensatory increase in HDL holo-particle uptake. However, the molecular mechanism or mechanisms that mediate the increase in adrenal HDL particle uptake has to be defined in the future.

This study and other experiments[17,23] reveal that in the complete absence of hepatic SR-BI, virtually no HDL selective CE uptake can be detected in the liver. Mice with an attenuated expression of SR-BI in the liver demonstrate a reduced hepatic selective CE uptake.[8] These observations provide evidence that SR-BI is the major molecule that mediates HDL selective CE uptake in vivo by the liver. However, in adrenals an SR-BI–independent mechanism for selective CE uptake is observed. In contrast, using radiolabeled human HDL, no SR-BI–independent selective CE uptake by adrenals was detected.[23] These differences in selective CE uptake by SR-BI–deficient glands may be explained by technical variations, ie, human HDL[23] versus murine HDL. In vitro studies using lipoprotein lipase and hepatic lipase suggest a mechanism for selective HDL CE uptake that is distinct from SR-BI.[10,11] Therefore, several lines of evidence suggest pathways for selective lipid uptake that are independent from SR-BI. However, at present the molecules involved in this process are not defined. Previously, it was proposed that protein-independent lipid–lipid interactions[12] may play a role in this metabolic route. However, future studies have to define an SR-BI–independent mechanism in more detail.

References

1. Tall AR. Plasma high density lipoproteins. Metabolism and relationship to atherogenesis. *J Clin Invest*. 1990;86:379–384.
2. Von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis. *Arterioscler Thromb Vasc Biol*. 2001;21:13–27.
3. Glass C, Pittman RC, Civen M, Steinberg D. Uptake of high-density lipoprotein-associated apoprotein A-I and cholesterol esters by 16 tissues of the rat in vivo and by adrenal cells and hepatocytes in vitro. *J Biol Chem*. 1985;260:744–750.
4. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science*. 1996;271:518–520.
5. Rinninger F, Pittman RC. Regulation of the selective uptake of high density lipoprotein-associated cholesteryl esters. *J Lipid Res*. 1987;28:1313–1325.
6. Wang N, Arai T, Ji Y, Rinninger F, Tall AR. Liver-specific overexpression of scavenger receptor BI decreases levels of high density lipoprotein in transgenic mice. *J Biol Chem*. 1998;273:32920–32926.
7. Rigotti A, Trigatti BL, Penman M, Rayburn H, Herz J, Krieger M. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor class B type I reveals its key role in HDL metabolism. *Proc Natl Acad Sci U S A*. 1997;94:12610–12615.
8. Varban ML, Rinninger F, Wang N, Fairchild-Huntress V, Dunmore JH, Fang Q, Gosselin ML, Dixon KL, Deeds JD, Acton SL, Tall AR, Huszar D. Targeted mutation reveals a central role for SR-BI in hepatic selective uptake of high density lipoprotein cholesterol. *Proc Natl Acad Sci U S A*. 1998;95:4619–4624.
9. Wang N, Weng W, Breslow JL, Tall AR. Scavenger receptor BI (SR-BI) is up-regulated in adrenal gland in apolipoprotein A-I and hepatic lipase knock-out mice as a response to depletion of cholesterol stores. *J Biol Chem*. 1996;271:21001–21004.
10. Bamberger M, Glick JM, Rothblat GH. Hepatic lipase stimulates the uptake of high density lipoprotein cholesterol by hepatoma cells. *J Lipid Res*. 1983;24:869–876.
11. Rinninger F, Brundert M, Brosch I, Donarski N, Budzinski RM, Greten H. Lipoprotein lipase mediates an increase in selective uptake of HDL-associated cholesteryl esters by cells in culture independent of scavenger receptor BI. *J Lipid Res*. 2001;42:1740–1751.
12. Morrison JR, Silvestre MJ, Pittman RC. Cholesteryl ester transfer between high density lipoprotein and phospholipid bilayers. *J Biol Chem*. 1994;269:13911–13918.
13. Pittman RC, Taylor CA Jr. Methods for assessment of tissue sites of lipoprotein degradation. *Methods Enzym*. 1986;129:612–628.
14. Silver DL, Wang N, Tall AR. Defective HDL particle uptake in ob/ob hepatocytes causes decreased recycling, degradation, and selective lipid uptake. *J Clin Invest*. 2000;105:151–159.
15. Rinninger F, Brundert M, Jäckle S, Galle PR, Busch C, Izbicki JR, Rogiers X, Henne-Bruns D, Kremer B, Broelsch CE, Greten H. Selective uptake of high-density lipoprotein-associated cholesteryl esters by human hepatocytes in primary culture. *Hepatology*. 1994;19:1100–1114.
16. Le NA, Ramakrishnan R, Dell RB, Ginsberg HN, Brown WV. Kinetic analysis using specific radioactivity data. *Methods Enzym*. 1986;129:384–395.
17. Van Eck M, Twisk J, Hoekstra M, Van Rij BT, Van der Lans CAC, Bos ST, Kruijt JK, Kuipers F, Van Berkel TJC. Differential effects of scavenger receptor BI deficiency on lipid metabolism in cells of the arterial wall and in the liver. *J Biol Chem*. 2003;278:23699–23705.
18. Khoo JC, Pittman RC, Rubin EM. Selective uptake of HDL cholesteryl esters is active in transgenic mice expressing human apolipoprotein A-I. *J Lipid Res*. 1995;36:593–600.
19. Pittman RC, Knecht TP, Rosenbaum MS, Taylor CA Jr. A nonendocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. *J Biol Chem*. 1987;262:2443–2450.
20. Glass CK, Pittman RC, Keller GA, Steinberg D. Tissue sites of degradation of apoprotein A-I in the rat. *J Biol Chem*. 1983;258:7161–7167.
21. Husemann J, Silverstein SC. Expression of scavenger receptor class B, type I, by astrocytes and vascular smooth muscle cells in normal adult mouse and human brain and Alzheimer's disease brain. *Am J Pathol*. 2001;158:825–832.
22. Chinetti G, Gbaguidi FG, Griglio S, Mallat Z, Antonucci M, Poulain P, Chapman J, Fruchart JC, Tedgui A, Najib-Fruchart J, Staels B. CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. *Circulation*. 2000;101:2411–2417.
23. Out R, Hoekstra M, Spijkers JAA, Krujit JK, van Eck M, Bos IST, Twisk J, van Berkel TJC. Quantitative analysis in scavenger-receptor BI (SR-BI) knock-out mice indicates the essential role of SR-BI in the selective uptake of cholesteryl esters from HDL by the liver and the adrenals. *J Lipid Res*. 2004;45:2088–2095.

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Scavenger Receptor Class B Type I Mediates the Selective Uptake of High-Density Lipoprotein–Associated Cholesteryl Ester by the Liver in Mice

May Brundert, Anne Ewert, Joerg Heeren, Barbara Behrendt, Rajasekhar Ramakrishnan, Heiner Greten, Martin Merkel and Franz Rinninger

Arterioscler Thromb Vasc Biol. 2005;25:143-148; originally published online November 4, 2004;
doi: 10.1161/01.ATV.0000149381.16166.c6
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636

Methods

Mice

Mice with a null mutation in the SR-BI gene (SR-BI KO) were established by Dr. Monty Krieger and coworkers at the Massachusetts Institute of Technology, Cambridge, MA, USA (7). These animals initially were created on a mixed C57BL/6 x 129 genetic background. The rodents were supplied by The Jackson Laboratory, Bar Harbor, Maine, USA. The mouse strain background of the animals used in this study was at least 80 % C57BL/6. To avoid background strain differences, littermate controls were used in all experiments. For genotyping the presence of the targeted or WT SR-BI alleles in DNA extracted from tail biopsies was detected by PCR amplification using primer 1 (5'-TGAAGGTGGTCTTCAAG-AGCAGTCCT-3') in combination with either primer 3 (mutant-specific, 5'-GATTGGGAA-GACAATAGCAGGC-ATGC-3) or primer 2 (WT-specific, 5'-TATCCTCGGCAGACCTG-AGTCGTGT-3') (7). Phenotyping of the mice was done by plasma cholesterol analysis.

Mice had unlimited access to regular chow diet and water. Light cycle was from 7.00 a. m. to 7.00 p. m.

All animal studies were approved by the local committee on laboratory animal care.

Lipoprotein Preparation

HDL (d = 1.063-1.21 g/ml) was isolated from WT (WT-HDL) and in parallel from SR-BI KO (homozygous) mouse plasma (SR-BI KO-HDL) by sequential ultracentrifugation (8). Mice fasted at least 4.0 hours before blood harvest.

WT-HDL or SR-BI KO-HDL were radiolabeled with ^{125}I-Tyramine Cellobiose (^{125}I-TC) and [^{3}H]Cholesteryl Oleyl Ether ([^{3}H]CEt, Amersham Biosciences) as described (13). The specific activity of ^{125}I-TC-/[^{3}H]CEt-WT-HDL was for ^{125}I-TC 32 +/- 5 cpm/ng protein and for [^{3}H]CEt 24 +/- 4 (n = 9 preparations); for ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL, the

respective values were 36 +/- 4 and 21 +/- 2 (n = 9 preparations; differences for each isotope between both preparations were not statistically significant).

HDL Metabolism in Mice

Plasma HDL metabolism and tissue sites of HDL uptake were investigated as described (3, 5). Briefly, food was removed from mice at least 4.0 hours before tracer injection, and animals fasted throughout the 24 hour study period but had unlimited access to water. Only male mice were used in these experiments. ^{125}I-TC-/[^{3}H]CEt-WT-HDL or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL was injected at 9 a. m. in the tail veins and blood samples (30 µl) were drawn at 2 minutes, 30 minutes, 2 hours, 5 hours, 9 hours, 22 hours and 24 hours postinjection. Plasma samples were directly radioassayed for ^{125}I-TC and analyzed for [^{3}H]CEt after lipid extraction (5). Twenty-four hours after tracer injection, the animals were anaesthetized, humanely killed and the vasculature was immediately perfused with saline (50 ml per mouse). Finally organs were harvested, homogenized and radioassayed. Tissue content of ^{125}I-TC radioactivity was directly assayed and that of [^{3}H]CEt was analyzed by liquid scintillation counting after lipid extraction (5).

Mouse Hepatocyte Isolation

Hepatocytes were prepared from livers of mice by collagenase perfusion (14). After isolation, the cells were cultured (37 ° C, 2.0 hours) in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies) supplemented with foetal bovine serum (5 %, v/v, heat-inactivated, Life Technologies), penicillin (100 IU/ml, Life Technologies) and streptomycin (100 µg/ml, Life Technologies).

Uptake Assay for doubly radiolabeled HDL

Hepatocytes were preincubated (37 ° C, 20 minutes) in DMEM containing bovine serum albumin (BSA, 5 mg/ml, SIGMA) and antibiotics (see above) (15). Thereafter followed an incubation (37 ° C, 2 hours) in DMEM containing BSA (5 mg/ml), ^{125}I-TC-/[^{3}H]CEt-WT-HDL or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL. Finally this medium was aspirated, the cells were

washed (PBS, 3 x) and harvested by gentle treatment with trypsin/EDTA solution (Life Technologies). Cellular uptake of HDL-associated tracers was analyzed as described (15).

Miscellaneous

Total cholesterol, unesterified cholesterol, phospholipid and triglyceride were measured with enzymatic assays (Wako). Protein was analyzed as described (15). Plasma lipoproteins were fractionated by fast protein liquid chromatography (FPLC) (8). Finally the cholesterol concentration of each FPLC fraction was measured.

Statistics and Calculations

Values are means +/- SEM. Significance of differences was calculated using Student's t-test.

In mice, based on decay of HDL-associated ^{125}I-TC and [^{3}H]CEt, plasma fractional catabolic rates (plasma-FCR's) were calculated using a two-compartment model (16). To analyze the activities of various tissues in HDL uptake and to directly compare the rates of uptake of the protein and the CE component of labeled HDL in those tissues, organ-FCR's for both HDL tracers were determined (3). These organ-FCR's were calculated as plasma-FCR x fraction of total tracer recovered (%) in a specific organ. These organ-FCR's represent the fraction of the plasma pool of the traced HDL component cleared per hour by an organ (3).

For hepatocytes, uptake of ^{125}I-TC-/[^{3}H]CEt-HDL is shown in terms of apparent HDL particle uptake, expressed as HDL protein (3, 15). This is done to compare the uptake of both tracers on a common basis. Uptake of HDL holo-particles is represented by equal uptake of both tracers.

In Figures and Tables, ^{125}I represents ^{125}I-Tyramine Cellobiose, [^{3}H] shows [^{3}H]Cholesteryl Oleyl Ether and [^{3}H] – ^{125}I indicates the difference, i. e. HDL selective CE uptake.

Results (supplement)

Uptake of both ^{125}I-TC-/[^{3}H]CEt-WT-HDL (40 µg HDL protein/ml) and of ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL (40 µg HDL protein/ml) by hepatocytes isolated from WT or from SR-BI KO (homozygous) mice was investigated (data not shown). In hepatocytes prepared from WT or from SR-BI KO mice and incubated (37 ° C, 2.0 hours) in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL, apparent HDL selective CE uptake ([^{3}H]CEt – ^{125}I-TC) was 1,174 +/- 78 or 151 +/- 38 ng HDL protein/mg cell protein, respectively (n = 6 experiments per group, $p < 0.0001$). In hepatocytes isolated from WT or from SR-BI KO mice which were incubated in medium containing ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL, apparent HDL selective CE uptake was 786 +/- 82 or 38 +/- 8 ng HDL protein/mg cell protein (n = 6 experiments per group, $p < 0.0001$). Thus when SR-BI-deficient hepatocytes incubated in medium containing ^{125}I-TC-/[^{3}H]CEt-WT-HDL or ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL, a significantly reduced hepatocellular selective CE uptake could be observed in vitro compared to WT liver cells.

Acknowledgements

This study was funded by grants from Deutsche Forschungsgemeinschaft (DFG), Bonn (Ri 436/8-1) and Werner Otto Stiftung, Hamburg, Germany.

Assistance by D. Greene, J. Greeve, B. Keller, P. Linsel-Nitschke, R. Morton, A. Rigotti, B. Schulz, D. Siver, A. Tall and M. Thiel is gratefully appreciated.

Table I

Plasma decay of ^{125}I-TC-/[^{3}H]CEt-WT-HDL in WT mice or of ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL in SR-BI KO mice and tracer uptake rates by tissues

^{125}I-TC-/[^{3}H]CEt-WT-HDL was injected in WT mice and in parallel ^{125}I-TC-/[^{3}H]CEt-SR-BI KO-HDL was injected in SR-BI KO (homozygous) animals. During the subsequent 24 hours blood was harvested periodically to determine the plasma decay of both tracers. Finally the animals were humanely killed and tissues were analyzed for the content of both tracers. Plasma-FCR's and organ-FCR's for ^{125}I-TC (^{125}I) and [^{3}H]CEt ([^{3}H]) were calculated as outlined in Methods. Values are means +/- SEM of n = 17 mice in each group. (*) $p < 0.005$.

Table I

	WT Mice			SR-BI KO Mice		
	Cholesterol	**^{125}I**	**[^{3}H]**	**Cholesterol**	**^{125}I**	**[^{3}H]**
Plasma Cholesterol (mg/dl)	98.5 ± 3.6*			190.5 ± 7.7*		
Plasma-FCR's (Pools/h)		0.0914 ± 0.0036	0.162 ± 0.0063*		0.0871 ± 0.0036	0.0752 ± 0.0031*
Organ-FCR's (x h^{-1} x 10^{3})						
Liver		32.0 ± 1.78	101.0 ± 4.3*		31.4 ± 1.57	31.6 ± 2.0*
Adrenals		0.139 ± 0.022*	0.430 ± 0.061*		0.250 ± 0.026*	0.226 ± 0.019*
Kidneys		3.84 ± 0.31	0.900 ± 0.06		4.07 ± 0.28	0.749 ± 0.07
Spleen		0.861 ± 0.049*	1.63 ± 0.104*		1.32 ± 0.084*	1.22 ± 0.077*
Stomach		1.33 ± 0.21	1.15 ± 0.17		1.14 ± 0.13	1.13 ± 0.14
Intestine		7.72 ± 0.62	4.66 ± 0.28		6.75 ± 0.54	4.06 ± 0.31
Brain		0.0373 ± 0.003	0.0651 ± 0.006*		0.0393 ± 0.004	0.0412 ± 0.0046*
Heart		0.513 ± 0.025	0.447 ± 0.023		0.581 ± 0.036	0.379 ± 0.031
Lungs		0.818 ± 0.50	1.44 ± 0.15*		0.718 ± 0.082	0.678 ± 0.081*
Carcass		44.2 ± 1.7	50.6 ± 3.9*		40.8 ± 1.6	34.9 ± 2.1*