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Albumin turnover: FcRn-mediated recycling saves as much albumin from degradation as the liver produces

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Kim, Jonghan, C. L. Bronson, William L. Hayton, Michael D. Radmacher, Derry C. Roopenian, John M. Robinson, and Clark L. Anderson. Albumin turnover: FcRn-mediated recycling saves as much albumin from degradation as the liver produces. *Am J Physiol Gastrointest Liver Physiol* 290: G352–G360, 2006. First published October 6, 2005; doi:10.1152/ajpgi.00286.2005.—It is now understood that the nonclassical major histocompatibility complex-I molecule FcRn binds albumin and retrieves it from an intracellular degradative fate. Whether FcRn in the liver modulates albumin turnover through effects on biosynthesis and production is not known. Thus we quantified the appearance of biosynthetically labeled albumin in plasma after an intravenous bolus injection of [^{3}H]leucine in FcRn-deficient mice. The production rates for both albumin (FcRn substrate) and transferrin (nonsubstrate) are increased by ~20% in FcRn-deficient mice compared with normal mice, likely compensating for the lowered plasma oncotic pressure caused by hypoalbuminemia in FcRn-deficient mice. Determining the magnitude of FcRn-mediated effects on albumin turnover, we then measured the steady-state plasma concentrations of biosynthetically labeled albumin and transferrin during [^{3}H]leucine infusion. The concentration of albumin was ~40% lower in FcRn-deficient mice compared with normal mice. Furthermore, the ~40% lower plasma albumin concentration in FcRn-deficient mice along with the ~20% increase in albumin production indicate, by the mass-balance equation, that albumin degradation in FcRn-deficient mice is twice that of normal mice. These studies of biosynthetically labeled, and thus native, albumin support our previous finding that FcRn protects albumin from degradation. Permitting quantification of the magnitude of FcRn-mediated recycling, they further indicate that FcRn has extraordinary capacity: the amount of albumin saved from degradation by FcRn-mediated recycling is the same as that produced by the liver.

production; clearance; steady-state; kinetics; Fc receptor

ALBUMIN and IGG, the two most abundant proteins in plasma, bind at low pH with high affinity to independent sites on the major histocompatibility complex-related Fc receptor (FcRn). Situated in acidic endosomes of virtually all nucleated cells, FcRn binds both ligands after they have been constitutively endocytosed, shunting them back to the pH-neutral cell surface for continuing circulation, thereby rescuing them from their usual lysosomal degradation fate. FcRn-deficient mice that lack such an FcRn-mediated recycling process (13) show shorter half-lives and lower steady-state plasma concentrations (Css) of both proteins than do normal mice (8).

From our earlier measurements of radioiodinated albumin clearance (CL) and endogenous Css, we had inferred that the production rate (Rp) of albumin, using the mass-balance equation (Rp = CL × Css), was lower in the FcRn knockout (KO) strain compared with the wild-type (WT) strain (8). However, we acknowledged the estimations of CL for radioiodinated albumin to be only qualitative, for such measurements for albumin have historically been fraught with imprecision due to structural and/or functional alterations induced by purification and iodination (32). In fact, we had anticipated finding a greater albumin Rp in KO mice, for it had long been known that hypoalbuminemia induces an upregulation of the synthesis of albumin and other proteins (6, 12, 23, 26, 33).

To understand more completely the role of FcRn on albumin turnover, we pursued three objectives in the present study. First, we measured albumin Rp directly by biosynthetic labeling with [^{3}H]leucine and determined the KO-to-WT ratio of Rp for albumin between the two strains to ascertain whether FcRn is involved in albumin production. Our results indicated that albumin Rp was enhanced in FcRn-deficient mice. Second, using biosynthetically labeled proteins, we compared albumin Css between KO and WT mice. The results confirmed the previous findings that FcRn plays an important role in maintaining plasma albumin concentration. These differences in the production and the plasma concentration of albumin between the two strains are highly quantitative because two different methods of measurement were applied. Finally, we computed the KO-to-WT ratio of CL between the two strains under steady-state conditions. This quantitative approach showed that the amount of albumin saved from a degradative fate by FcRn-mediated recycling was equivalent to the amount of albumin produced by the liver.

MATERIALS AND METHODS

Animals. FcRn α-chain KO mice (B6.129X1/SvJ*Fcgrt*^{Tm1Dcr}) (29) and their control WT mice (C57BL/6J) were kindly provided by the Roopenian laboratory at The Jackson Laboratory (Bar Harbor, ME). All mice were male and ~3–4 mo old. The Institutional Review Board approved all animal studies.

Css of plasma proteins in FcRn-KO and WT mice. Css of endogenous albumin and transferrin were measured using sandwich ELISA (8). We found higher transferrin concentrations (Table 1) than has been typically seen (<5 mg/ml) by others (3, 4, 27, 28). Nevertheless, the interpretation of our studies would not be altered by lower absolute values because we make use of ratios of transferrin concentrations between KO and WT mice. The differences may be due to assay differences in either the transferrin standard or the antibody specificity. Neither was well characterized by the other workers. The

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Table 1. *Physiological quantities of WT and FcRn KO mice*

	WT	KO	KO/WT (unitless)	*P* Value
Weight				
Body, g	28.7±1.92	28.0±2.05	0.976	0.492
Liver, g	1.27±0.0735	1.19±0.111	0.937	0.109
Liver/body wt, %	4.43±0.271	4.24±0.160	0.957	0.113
Plasma				
Albumin, mg/ml	36.3±1.23	21.0±1.20	0.579	4.57×10^{-13}
Transferrin, mg/ml	8.71±0.926	10.9±0.650	1.25	1.33×10^{-4}
SAP, μg/ml	5.95±1.77	6.50±1.36	1.09	0.501
Total protein, mg/ml	50.3±1.38	42.9±1.34	0.853	3.74×10^{-8}
Liver				
Total protein, mg/g liver	231±10.5	230±9.94	0.996	0.851

Values are means ± SD. Physiological quantities and endogenous, steady-state protein concentrations (Css) in plasma and the liver were measured and determined in untreated wild-type (WT) and knockout (KO) mice (4-mo-old, male, $n = 8$, each strain). The *P* value between the 2 strains is based on the 2-tailed Student's *t*-test.

high degrees of specificity and sensitivity of our ELISA and our antibodies against albumin and transferrin have been documented previously (15). Total plasma protein concentration was estimated by the bicinchoninic acid (BCA; Pierce, Rockford, IL) method against a BSA standard solution.

Plasma albumin and transferrin after [^{3}H]leucine bolus injection. KO and WT mice were injected via the tail vein with 50 μCi/mouse of [^{3}H]leucine ([4,5-^{3}H]leucine; 156 Ci/mmol; Amersham, Piscataway, NJ). Blood (30 μl) was collected with heparinized capillary tubes from the retroorbital plexus at 15, 30, 45, and 60 min after the intravenous bolus injection of [^{3}H]leucine, and plasma was harvested and stored at −80°C. At 90 min, the mice were exsanguinated, and plasma was stored at −80°C.

Three microliters of plasma collected at each time point were subjected to SDS-PAGE under reducing conditions with 2% (vol/vol) 2-mercaptoethanol. Uniform gels (10%) were prepared; each gel contained two plasma samples (2 wells/gel). The gels were run for 1.5 h, stained with Coomassie blue G for 20 min, and destained for 30 min under 10% acetic acid. After the gels were washed with water, the albumin and transferrin bands were excised carefully by scalpel and blade followed by overnight digestion at room temperature with 2 ml of tissue gel solubilizer (NCS tissue solubilizer; Amersham) to extract proteins. Two milliliters of Ecolite (MP Biomedicals, Irvine, CA), 14 ml of BCS cocktail (Amersham), and 80 μl of acetic acid were added sequentially to the digested bands, and the radioactivity was determined by liquid scintillation counting (LSC; Beckman, Fullerton, CA). ^{3}H-labeled protein concentrations in the plasma were normalized to a dose per body weight of 50 μCi/25 g.

Immunoadsorption (IA) was employed as an additional method for measuring the appearance rate of albumin and transferrin, modified from Ruot et al. (31) with ^{3}H-labeled plasma from KO and WT mice at 90 min after administration of [^{3}H]leucine. Protein G-Sepharose (100 μl; ~18 mg human IgG/ml drained beads; Amersham) was preequilibrated with Tris-buffered saline (TBS) and incubated with 100 μl (1 mg/ml) of affinity-purified goat IgG against albumin or transferrin (Bethyl, Montgomery, TX) diluted in 200 μl of TBS for 1 h at 4°C in an Ultrafree-MC centrifugal filter unit (Millipore, Billerica, MA) with gentle rocking. After the beads were washed four times with TBS containing 0.05% Tween 20, ^{3}H-labeled plasma from KO and WT mice (0.5 μl for albumin or 2.5 μl for transferrin) at 90 min after administration of [^{3}H]leucine was diluted in TBS containing 0.05% Tween 20 (300 μl), added to the beads, and incubated for 3 h at 4°C with gentle rocking. A preclearing step was unnecessary because there was no significant adsorption of an irrelevant antibody (rabbit anti-ovalbumin antibody; Sigma, St. Louis, MO). The beads were extensively washed, solubilized by tissue solubilizer, and radioactivity was determined by LSC.

The validity of the two methods applied in the present study has been documented previously (15). Radiolabeled albumin and transferrin were separated by SDS-PAGE and IA with a high recovery (>85%) and purity (>95%). The two methods also provided high degrees of linearity ($R^2 > 0.9974$) and reproducibility (coefficient of variation < 8%). Antibody against mouse serum albumin or transferrin was specific in its binding to albumin or transferrin.

Plasma albumin and transferrin after [^{3}H]leucine infusion. [^{3}H]leucine (250 μCi/mouse) was infused for 2 wk via subcutaneously implanted osmotic pumps (100-μl size; Alzet, Cupertino, CA) into KO and WT mice to establish the Css of biosynthetically labeled albumin and transferrin. On the basis of general kinetic principles, the time to reach steady state of a substance is not a function of the rate of infusion but of elimination (11). Furthermore, from the start of infusion, the plasma concentration of a substance increases over time and reaches a plateau (~97% of its Css) in five half-lives (30). Hence, the Css of leucine, the half-life of which is <15 min in rats (24), can be reached after approximately five times its half-life, which is <1.5 h. As an essential amino acid, leucine absorption into the systemic circulation is rapid. The 1.5-h period is quite short compared with a 14-day infusion. However, for albumin and transferrin, with half-lives of ~1.5–2 days, we had to study >10 days to reach their steady-state concentrations. Certainly, leucine must be at steady state for the proteins to be at steady state. For this reason, we infused for 2 wk and achieved steady state for both proteins (see RESULTS). According to the pump supplier's specifications, the average infusion rate was 0.21 ± 0.01 μl/h and the averaged dosing rate was 0.525 ± 0.025 μCi/h. Specifically, osmotic pumps were preequilibrated for 4 h in 0.9% isotonic saline solution at 37°C and released leucine at a constant rate before subcutaneous implantation between the scapulae of mice using aseptic technique. Blood (~30 μl) was taken at *days 7, 10, 11, 12, 13,* and *14* from the retroorbital plexus under light isoflurane anesthesia, and the mice were exsanguinated from the inferior vena cava at *day 14*. The liver was excised quickly and weighed, and plasma was collected by centrifugation. Plasma, liver, and dosing solution samples were stored at −80°C. Osmotic pumps were recovered, and the remaining leucine was collected to check for adequate release of leucine over the course of the experiment.

Steady-state plasma concentrations of biosynthetically labeled and endogenous albumin and transferrin in KO and WT mice were determined using SDS-PAGE and ELISA, respectively. The radioactivities of albumin and transferrin in the plasma were measured by SDS-PAGE followed by LSC, and the steady-state plasma concentrations of the endogenous proteins (labeled and unlabeled) were determined by ELISA. The radioactivity of liver protein was measured by LSC after trichloroacetic acid (TCA) precipitation (described in the following section). Radiolabeled albumin and transferrin concentrations were normalized to the same radiolabeled liver protein concentration at *day 14*.

Liver proteins. We considered whether a strain difference in the uptake of radioactive leucine or leucine metabolism caused a prefer-

ential availability of precursor in the liver between the two strains. Because the true precursor for protein synthesis is not the amino acid itself but an aminoacyl-tRNA, which is difficult to characterize, we used a more easily measurable marker for precursor availability. Because plasma albumin and transferrin are produced exclusively in the liver, liver total protein would share the same precursor with albumin and transferrin; thus the magnitude of liver total protein synthesis would be a suitable surrogate marker for precursor availability for albumin and transferrin production. In addition, in the preliminary experiment we measured the steady-state leucine concentration in the plasma in both strains using reversed-phase HPLC by a postderivatization technique and found no substantial difference between the two strains (not shown).

For liver protein synthesis, KO and WT mice were injected with 50 μCi/mouse of [^{3}H]leucine. At 30 min after the injection, mice were exsanguinated, and liver was excised and homogenized (Polytron; Brinkmann, Westbury, NY) in four volumes of ice-cold TBS containing 1.25% protease inhibitor cocktail (P-8340, Sigma). TCA (20%, 200 μl) was added to 200 μl of liver homogenate to precipitate the proteins. The pellet was dissolved in 0.1 N NaOH overnight, and the radioactivity was measured, and [^{3}H]protein concentrations in the liver were normalized to 50 μCi/25 g body wt. Total protein concentrations in liver homogenates were estimated by the BCA method.

Biosynthesis of albumin and transferrin. In the context of this study, production describes exocytosis or secretion of biosynthesized protein, whereas biosynthesis describes the events before secretion. In assessing albumin turnover, it is imperative that we measure production; a measurement of biosynthesis may become important when a receptor is involved before secretion. To quantify the rate of appearance of biosynthetically labeled albumin in the liver, KO and WT mice were injected with 50 μCi/mouse of [^{3}H]leucine. At 5, 7.5, and 10 min after the injection, five to seven mice of both strains were anesthetized and exsanguinated at each time point, and the liver was quickly excised and homogenized (Polytron) in two volumes of ice-cold TBS containing 1.25% protease inhibitor cocktail (P-8340). Immunoadsorption was applied to quantify the biosynthesis of albumin and transferrin in the liver. Radioactivities of albumin and transferrin in the liver, normalized to the total radioactivity of liver protein, were compared between KO and WT mice. In addition, because transferrin is not a substrate for FcRn, a ratio of albumin appearance to transferrin appearance in the liver was calculated in both KO and WT mice. The two ratios were then compared to ascertain whether presecretory degradation in the liver exists in the absence of FcRn.

Plasma serum amyloid P. Plasma serum amyloid P (SAP) concentration was measured by sandwich ELISA (7). The acute-phase reaction was induced by nonaseptic, subcutaneous implantation of an osmotic pump and by intermittent bleedings from the retroorbital plexus of KO and WT mice. Plasma was collected at 0 (before implantation), 6, 12, and 18 h after implantation.

Rp of albumin and transferrin. Because our primary objective was to compare the Rp of albumin between the two strains, the equation (2, 20) for calculating Rp was simplified

$$\mathrm{Rp} = \frac{\mathrm{C_T} \times \mathrm{V_1} + k \times \int_0^T \mathrm{C_t} dt \times \mathrm{V_1}}{n \times \mathrm{sa_{Leu}} \times 1.5\ \mathrm{h}}$$

where C_T [10^3 disintegrations·min^{-1}·ml plasma^{-1} (kdpm/ml plasma)] and C_t represent the plasma concentrations of ^{3}H-labeled protein at *time T* (= 90 min) at which the maximum concentration of labeled protein is achieved and at indefinite *time t* (from *time 0* to *T*), respectively, V_1 is the volume of the initial distribution compartment of protein, k is the protein disappearance rate constant (1, 18), n is milligrams of leucine per milligram of albumin or transferrin, and sa_{Leu} represents the averaged precursor specific activity of [^{3}H]leucine (truly, [^{3}H]aminoleucyl-tRNA) in the liver during 90 min, which was reflected by and proportional to the total ^{3}H-labeled protein synthesis in the liver at 30 min (see DISCUSSION). The units of Rp are kdpm per hour per unit precursor (relative Rp), which is convertible to milligrams per hour per gram of liver (absolute Rp) if the specific activity of leucine is provided (unknown in this comparative study).

The numerator describes the amount of newly produced protein appearing in the plasma during 90 min as the sum of the amount present at 90 min ($C_{90} \times V_1$) plus the amount of protein that was lost ($k \times \int C_t dt \times V_1$) during the period. The denominator describes the average magnitude of the precursor pool for the proteins.

CL of albumin and transferrin. CL was calculated by rearrangement of the mass-balance equation (CL = Rp/Css). Because Rp was a relative magnitude in the absence of an absolute value for the precursor pool, the calculated CL was also a relative magnitude but comparable between the two strains. Two CL values were computed based on SDS-PAGE and antibody-based assays (IA and ELISA), because each method generated an Rp and Css for the proteins. The ratio of CL between KO and WT strains was used as a quantitative measure of FcRn-mediated recycling of albumin. The KO-to-WT ratio for transferrin CL was used as a control.

FcRn-mediated recycling rates of albumin and IgG. Using the mass-balance equation under steady-state conditions where the production rate equals the catabolic rate, we calculated the FcRn-mediated recycling rate as the product of the CL differences between KO and WT mice and the plasma Css of the WT strain. Turnover kinetics of both FcRn ligands, albumin and IgG, were determined and are expressed in Fig. 4. Specifically, Css of albumin and IgG were measured by ELISA. Although absolute CL values were not computed in the present study, we calculated an accurate albumin CL ratio between KO and WT mice. Knowing the CL value of KO mice allows identification of the CL of WT mice. Radioiodinated albumin appears to be ineffectively recycled by FcRn, possibly due to chemical/structural alterations (see DISCUSSION). Therefore, while the exact CL value for albumin in KO mice was taken from previous studies (8), albumin CL in WT mice was computed using the CL ratio between KO and WT mice measured using biosynthetically labeled albumin. Because IgG biosynthesis is impossible to measure due to its much lower Css (compared with albumin and transferrin), the absolute CL values for IgG were calculated from the radioiodinated IgG decay curve (Ref. 8 and C. L. Bronson, unpublished data). Thus the comparison of kinetic parameters between albumin and IgG (see Fig. 4) should be considered semiquantitative. The rate of production, identical to the rate of catabolism at the steady state in the physiological condition, was calculated as the product of CL and Css (the mass-balance equation).

Statistical analyses. The two-tailed Student's *t*-test was applied to compare the protein concentrations and kinetic parameters between KO and WT mice. Differences between the two strains were considered statistically significant at $P < 0.05$. Data are presented as averages ± SD.

In the experiment to determine Css of albumin and transferrin, linear mixed-effects models were fitted for the SDS-PAGE and ELISA data to assess the fixed effect of mouse strain on Css after controlling for repeated measurements made after steady state was reached (*days 11-14*). Differences in protein concentration between KO and WT strains were considered significant at $P < 0.05$.

A bootstrap analysis (10) was used to assess the difference in the KO-to-WT ratio of Css between SDS-PAGE and ELISA for albumin and transferrin. If no difference existed between the two methods, we expected the KO-to-WT ratio for SDS-PAGE to equal the KO-to-WT ratio for ELISA; the ratio of ratios should equal unity. Analyses were performed using the R statistical software language (http://www.R-project.org).

RESULTS

Steady-state protein concentrations in plasma and the liver. Endogenous, or physiological, steady-state protein concentrations in the plasma and liver of untreated KO and WT strains are listed in Table 1. Plasma concentrations of albumin, transferrin, and total protein in KO strains were significantly different ($P < 0.001$) compared with WT strains, i.e., 42% lower, 25% higher, and 15% lower Css for albumin, transferrin, and total plasma proteins, respectively. SAP, a marker for the acute-phase reaction, did not differ between the two strains. The body and liver weights and the total protein concentration in the liver were not different between the two strains, and there was no detectable albuminuria or proteinuria in either mouse strain.

Plasma [^{3}H]albumin and [^{3}H]transferrin after [^{3}H]leucine bolus injection. Figure 1 shows the plasma appearance of newly produced [^{3}H]albumin and [^{3}H]transferrin in KO and WT strains after protein separation by SDS-PAGE. After intravenous injection of [^{3}H]leucine, the plasma appearance of both proteins, determined at the early time points, was not different between the two strains, whereas at 45 min and beyond, the KO mice showed a greater rate of plasma appearance of both albumin and transferrin than did the WT mice. At 60–90 min, the WT mice approached a plateau level in the appearance of both albumin and transferrin, and the KO mice showed the continuing appearance of both proteins in the plasma. A preliminary study showed that while the concentrations of the ^{3}H-labeled proteins increased slightly between 90 min and 3 h, the ratio of [^{3}H]albumin concentration at 3 h between the two strains was identical to that at 90 min (not shown); the concentration was ~27% higher in KO compared with WT mice. Transferrin showed the same kinetics and magnitude of increase in the ratio (~25%).

The radioactivity associated with albumin and transferrin measured by IA was also significantly higher ($P < 0.05$) in KO compared with WT plasma at 90 min, showing ~17% and ~15%, respectively, increases in KO compared with WT mice. The results of the SDS-PAGE and IA were consistent in that rates of plasma appearance of both albumin and transferrin were increased in KO compared with WT mice.

Plasma [^{3}H]albumin and [^{3}H]transferrin after [^{3}H]leucine infusion. Figure 2 shows the plasma concentration of ^{3}H-labeled (Fig. 2*A*) and endogenous (Fig. 2*B*) albumin and transferrin during [^{3}H]leucine infusion at a constant rate over 2 wk into KO and WT mice via subcutaneously implanted osmotic pumps. Both [^{3}H]albumin and [^{3}H]transferrin reached steady state because their plasma concentrations were not different during *days 11–14*. Endogenous albumin and transferrin were also at steady state. The albumin steady-state concentration (averaged over all steady-state measurements) in KO mice was 33% lower than the concentration in WT mice (218 vs. 323 kdpm/ml) by SDS-PAGE (as [^{3}H]albumin) and was 44% lower than the concentration in WT mice (21.4 vs. 38.5 mg/ml) by ELISA (as endogenous albumin). These differences in Css between the two strains were statistically significant by linear mixed-effect analysis, and, by the bootstrap analysis, the KO-to-WT ratios of Css between SDS-PAGE and ELISA were significantly different. The concentrations of both ^{3}H-labeled (54.0 vs. 48.5 kdpm/ml) and endogenous (11.0 vs. 9.86 mg/ml) transferrin in KO mice were 11% higher (statistically different by linear mixed-effect analysis) than the corresponding concentrations in WT mice.

Plasma SAP. To determine whether the acute-phase response might alter our results, we first characterized the kinetics of SAP in both strains after a harsher surgical procedure than experiments for Rp and Css. Plasma concentrations of SAP, a surrogate for the acute-phase response, remained baseline (~7 μg/ml) until 6 h, began to increase to plateau after 12 h, and reached values near 100 μg/ml at 18 h (Fig. 3). We then measured SAP concentration during the [^{3}H]leucine bolus experiment. There was a significant difference in SAP level between KO and WT mice at both 15 min (7.65 vs. 5.75 μg/ml) and 90 min (7.35 vs. 5.70 μg/ml) after [^{3}H]leucine. However, not only were SAP concentrations in both strains well within the basal level (~25 μg/ml) of WT (C57BL/6J) strains (22), but there was no significant difference in SAP level between the two time points (from 15 to 90 min) either in KO or in WT mice, and the experiment was finished (90 min) before the onset of the acute-phase response (6 h). Low SAP levels were also maintained in the [^{3}H]leucine infusion experiment (*days 7–14*). These data suggested that the acute-phase response was insignificant in our measurements of Rp and Css.

Liver proteins. To assess whether the [^{3}H]leucine pool for biosynthetic labeling of albumin and transferrin differed between the two strains, we measured total liver ^{3}H-labeled protein synthesis and steady-state liver protein concentration in

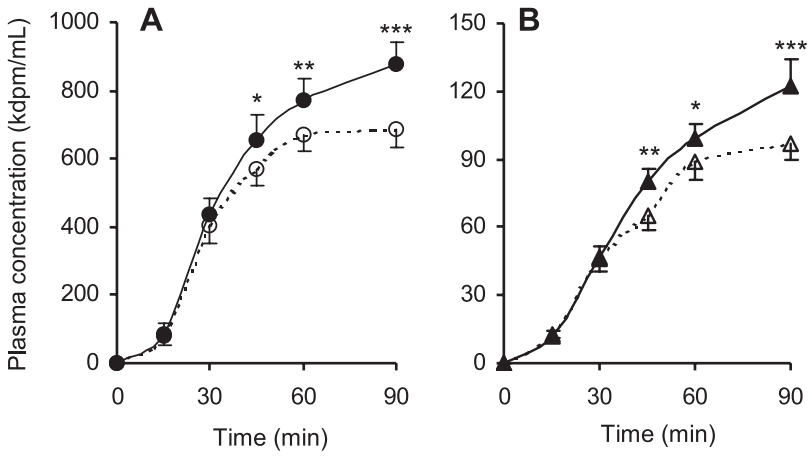

Fig. 1. Plasma appearance of [^{3}H]albumin and [^{3}H]transferrin after an intravenous bolus injection of [^{3}H]leucine in wild-type (WT) and knockout (KO) mice. Plasma concentrations of [^{3}H]albumin (*A*; circles) and [^{3}H]transferrin (*B*; triangles) over time, measured by SDS-PAGE, from WT (open symbols; $n = 7$) and KO (filled symbols; $n = 7$) mice were normalized to the radioactivity dose per body wt (50 μCi/25 g). kdpm/ml, 10^3 disintegrations·min^{-1}·ml plasma^{-1}. Significant statistical differences between the 2 strains at individual time points are designated as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$ derived from the Student's *t*-test. Error bars represent 1 SD.

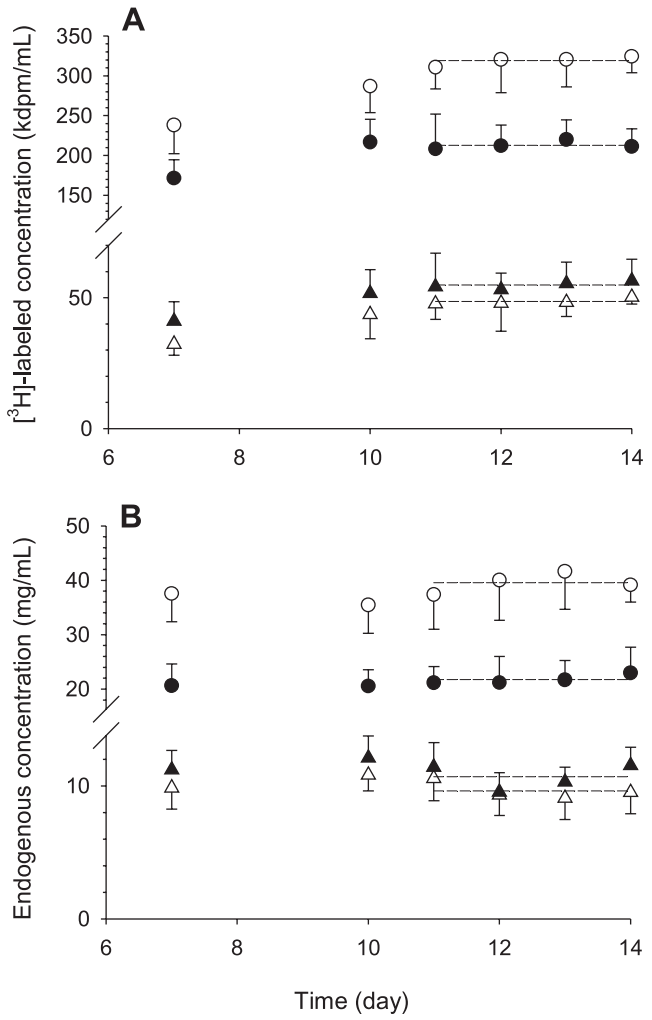

Fig. 2. Steady-state plasma concentration of albumin and transferrin after [^{3}H]leucine infusion in WT and KO mice. Steady-state plasma concentrations of albumin (circles) and transferrin (triangles) from WT (open symbols; $n = 7$) and KO (filled symbols; $n = 10$) mice were determined by 2 different methods: antibody-independent (SDS-PAGE; *A*) and antibody-dependent (ELISA; *B*), during a 14-day infusion of [^{3}H]leucine via subcutaneously implanted osmotic pumps. *A*: plasma steady-state concentrations of [^{3}H]albumin and [^{3}H]transferrin of WT and KO mice measured by SDS-PAGE. Steady-state concentrations of [^{3}H]albumin and [^{3}H]transferrin were considered achieved at *days 11–14* based on the linear mixed-effects analysis (see RESULTS). The dashed lines indicate the averaged values of protein levels from *days 11–14* as plasma steady-state concentrations. *B*: endogenous (labeled and unlabeled) plasma concentrations of albumin and transferrin of WT and KO mice measured by ELISA.

KO and WT mice 30 min after an intravenous bolus injection of [^{3}H]leucine. Total liver protein concentration showed no difference between the two strains (225 vs. 230 mg/g liver; $P = 0.348$), consistent with the data from untreated animals (Table 1). Because blood retention is ~25% in the total wet liver (14, 34), the plasma contribution to liver weight would be ~14%, because the hematocrit of the mouse is typically ~45% (9). Therefore, the plasma protein contribution, ~7 mg/g liver, which accounts for <3% of total liver protein, was insignificant. As well, total liver protein concentration appears insensitive to the Rp of these two proteins, which likely constitutes a small fraction (~10%) of total liver protein biosynthesis (20, 25). The plasma appearances of these two proteins did not show divergence between strains until after 30 min, which is likely beyond the hepatic minimum transit times (MTT) for albumin and transferrin. MTT for albumin and transferrin are 16 and 30 min, respectively, in the rat (19, 25). The estimated MTT (Fig. 1), the intercept on the time axis of the rising phase of the plasma appearance curve, for [^{3}H]albumin and [^{3}H]transferrin were similar, ~10 min. We would infer, then, that molecules of [^{3}H]albumin and [^{3}H]transferrin nascent in the liver at 30 min would not appear in the plasma until ~40 min after [^{3}H]leucine injection, at which time KO mice showed a greater rate of plasma appearance of both proteins compared with WT mice (Fig. 1). Thirty minutes were also chosen to measure total liver protein synthesis because liver ^{3}H-labeled protein is near its highest plateau level, whereas plasma ^{3}H-labeled protein is minor (24). At later times, liver protein synthesis is not a valid marker for precursor availability because plasma ^{3}H-labeled protein retained in the liver increases (Fig. 1), whereas liver ^{3}H-labeled protein is greatly decreased. These considerations indicate that the increase in [^{3}H]albumin and [^{3}H]transferrin production in KO compared with WT mice (Fig. 1) cannot result from strain differences in the availability of precursors.

Biosynthesis and presecretory recycling of albumin. Because FcRn recycles albumin out of the cell, saving it from degradation, we asked whether liver FcRn mediates presecretory recycling of nascent albumin (not transferrin). We first observed that the liver-radiolabeled protein concentrations were almost identical between KO and WT mice at three different time points: 5, 7.5, and 10 min (not shown; $P = 0.7241$). Second, at all time points studied, we found that radioactivity of albumin normalized to liver-radiolabeled protein synthesis was not statistically different between the two strains (not shown; $P = 0.2132$). Transferrin showed the same pattern. Then, we compared the ratio of appearance of albumin to transferrin in the liver between KO and WT mice. The ratio

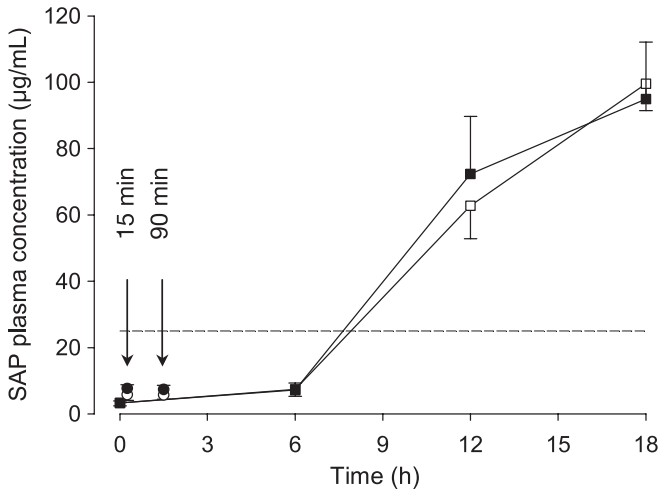

Fig. 3. Plasma serum amyloid P (SAP) in WT and KO mice. Plasma concentrations of SAP over the course of time in 2 experiments are shown. First, the plasma kinetics of SAP were characterized after the nonaseptic, subcutaneous implantation of osmotic pumps in WT (□) and KO (■) mice at *time 0* (untreated control), and after 6, 12, and 18 h. Solid lines connect between data. Then, plasma SAP concentrations were determined at 15 and 90 min in both WT (○) and KO (●) mice ($n = 7$, each strain) during the [^{3}H]leucine intravenous bolus experiment shown in Fig. 1. The dashed line shows the reported basal SAP level (~25 μg/ml) in the same C57BL/6J mice (22).

in KO mice was not significantly different from that in WT mice (11.3 ± 2.41 vs. 10.9 ± 2.78 at 5 min, 10.8 ± 1.66 vs. 11.8 ± 0.938 at 7.5 min, and 11.8 ± 3.41 vs. 13.9 ± 3.54 at 10 min; ratio KO vs. WT, averages ± SD; $n = 5$–7 at each time point), suggesting that there is no significant presecretory recycling of albumin in the liver in the presence of FcRn.

Rp of albumin and transferrin. Because the precursor pool for albumin production did not differ between the two strains, Rp values for albumin and transferrin were calculated and compared as the KO-to-WT ratio of Rp (Table 2). The disappearance rate constants (rate constants for distribution into extravascular compartment plus degradation) of albumin and transferrin in both KO and WT strains, calculated from the albumin and transferrin disappearance curve after intravenous bolus injection of biosynthetically labeled plasma (not shown) or radioiodinated albumin (C. L. Bronson, unpublished data), were not different (~0.00167/min during the first 3 h) between the two strains; the reference plasma volume (V_1) was also the same between the two strains (C. L. Bronson, unpublished data), and a value of 4% of body weight was used. Because the amount of protein lost by disappearance, shown as ($k \times \int C_t dt \times V_1$), was calculated to be ≤10% of total production ($C_{90} \times V_1 + k \times \int C_t dt \times V_1$) of both albumin and transferrin, the rate of plasma appearance of ^{3}H-labeled proteins accounted for ~90% of the Rp of both proteins. Therefore, strain differences in protein disappearance do not appear to influence the Rp measurement during the experimental period (Fig. 1). In KO compared with WT mice, the calculated Rp of albumin and transferrin was increased by 27% (639 vs. 505 kdpm·h^{-1}·unit precursor^{-1}) and 25% (88.1 vs. 70.7 kdpm·h^{-1}·unit precursor^{-1}), respectively, measured by SDS-PAGE and 17% (424 vs. 362 kdpm·h^{-1}·unit precursor^{-1}) and 15% (49.4 vs. 43.0 kdpm·h^{-1}·unit precursor^{-1}), respectively, measured by IA. All of the differences in Rp between KO and WT strains were significant.

In addition, while at later times we saw significantly different plasma concentrations of [^{3}H]albumin and [^{3}H]transferrin between the two strains, the concentrations at earlier times (15 and 30 min) were not different between the two strains. These changes with time would imply that along with a significant increase in the production rates of both proteins in the KO mice, there is also a substantial increase in the secretion (or exocytosis) time. This possibility, that increased amounts of plasma proteins produced in KO mice might prolong the time for secretion of the proteins, was suggested by others who showed that the transferrin secretion rate was accelerated from hepatocytes of analbuminemic rats compared with normal rats. These authors postulated a "competition" model in which transferrin has less competition for secretion from the negligible amount of albumin in analbuminemic rats (21).

Table 2. *Steady-state turnover parameters of albumin and transferrin and the effect of FcRn on albumin degradation*

	KO/WT (unitless)		
	Rp	Css	CL
Albumin			
SDS-PAGE	1.27	0.675	1.88
IA or ELISA	1.17	0.556	2.11
Transferrin			
SDS-PAGE	1.25	1.11	1.12
IA or ELISA	1.15	1.11	1.03

The production rate (Rp) was computed by the appearance of radiolabeled protein up to 90 min with a correction for plasma disappearance (hence, $C_{90} \times V_1 + k \times C_t dt \times V_1$) and divided by 90 min at an identical precursor pool measured by SDS-PAGE or immunoadsorption (IA) after intravenous bolus injection of [^{3}H]leucine. Css for ^{3}H-labeled and endogenous albumin and transferrin was determined by SDS-PAGE and ELISA, respectively. For individual magnitudes, see RESULTS. Two different forms of albumin clearance (CL) values were computed by the 2 methods and compared as the KO-to-WT ratio. CL = Rp/Css. Because the absolute magnitude of the precursor pool was not measured, calculated Rp and CL can be appreciated as relative values relating to absolute values for Rp and CL (see MATERIALS AND METHODS). The KO-to-WT ratio of CL indicates the contribution of FcRn in the CL, a measure of protein degradation.

Clearance of albumin and transferrin. The measured Css and Rp for albumin and transferrin were used to calculate CLs (CL = Rp/Css), expressing them as a ratio of KO to WT values (Table 2). Both methods of protein purification yielded similar KO-to-WT ratios of albumin CL, ~2, i.e., 1.88 by SDS-PAGE and 2.11 by antibody-dependent methods (IA and ELISA), indicating that albumin is degraded ~100% more rapidly in the FcRn-deficient mice than in the WT mice. This result not only supports our previous observation, using biosynthetically labeled native albumin, that FcRn protects albumin from degradation, but also allows us to accurately quantify the magnitude of its recycling, showing a large capacity of whole body FcRn for albumin. In contrast, transferrin, having no affinity for FcRn, showed essentially no difference in CL between the two strains; its KO-to-WT ratio was ~1.

DISCUSSION

In the present study, we calculated the albumin Rp directly by biosynthetically incorporating radiolabeled amino acid into the protein and measuring its appearance in plasma. This direct, quantitative approach would seem inherently more valid than our earlier estimation (8), which was based on a calculation of Rp using measurements of both the rate of plasma decay of injected radioiodinated albumin and endogenous plasma albumin Css. Although the Css value is relatively unimpeachable, CL or half-life, derived from the decay of radioiodinated purified albumin, is fraught with long demonstrated imprecision that likely relates to chemical alterations of the molecule (16, 32). Therefore, on the basis of our approach using biosynthetically labeled albumin, our earlier KO-to-WT ratio of CL derived from radioiodinated albumin decay (8) was underestimated (as was FcRn-mediated recycling), causing an overestimation of the Rp in the WT mice. Transferrin, chosen as a control protein, played two roles: it served as a negative control protein for FcRn-mediated processes in that it does not bind FcRn, but it was a positive control protein for hypoosmotic pressure-induced Rp upregulation effects caused by low albumin Css in KO strains.

Our finding that the Rp of both albumin and transferrin is increased in FcRn-deficient mice fits well the concept that hypoalbuminemia somehow induces an increase in plasma protein production (6, 12, 23, 26, 33). Furthermore, the disparity of a 15% decrease in the total plasma protein concentration despite a 42% decrease in albumin Css (Table 1) in the KO mice would suggest that in response to hypoalbuminemia, there is an upregulation of a variety of plasma proteins such as transferrin.

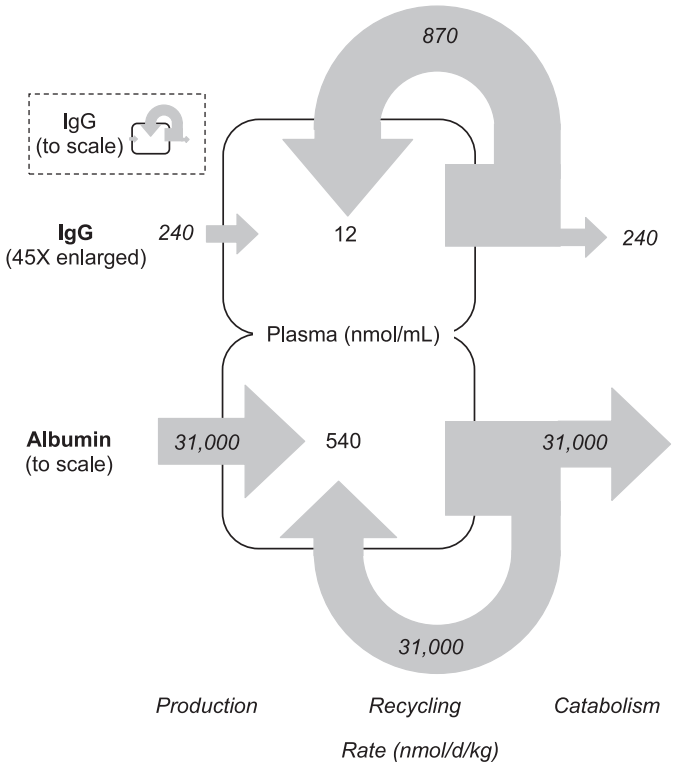

Fig. 4. Schematic representation of homeostasis of FcRn substrates IgG and albumin in the mouse at physiological concentrations. The turnover rates for albumin and IgG, the 2 FcRn substrates, are shown for a whole mouse represented as a single giant cell on a semiquantitative scale. While the *inset* (dotted box) shows the actual scale for IgG, a magnified (by 45-fold) figure is aligned to the actual scale for albumin. Shown are the plasma concentrations (542 vs. 12.1 nmol/ml; albumin vs. IgG), the catabolic rates to the *right* (30,700 vs. 240 nmol·day^{-1}·kg^{-1}), the FcRn-mediated recycling rates back to the plasma (30,700 vs. 868 nmol·day^{-1}·kg^{-1}), and the production rates from the *left* (see MATERIALS AND METHODS).

The differences between the two mouse strains appear to be highly significant because two different precise methods of measurement were employed: SDS-PAGE and immunoadsorption. Both methods were quantitative based on the high degrees of linearity and reproducibility (15). Both Rp and Css for albumin in the two strains varied in the same direction no matter which method was employed: KO > WT for Rp and KO < WT for Css by either method; transferrin showed KO > WT for Rp and KO > WT for Css by both methods.

On the basis of our study, there are two factors maintaining a high albumin plasma concentration: the liver and FcRn. Our result shows that the clearance for albumin in the KO mice is twice what it is in the WT mice; or, the FcRn-mediated recycling process saves as much albumin from degradation as is made in the liver. In graphic terms, a FcRn-deficient animal would need an additional liver as large as the existing one to maintain normal plasma albumin concentrations. One might also infer that the WT mice are producing albumin at a near-maximum rate, because albumin production increases by only ~20% in the KO mice to compensate for the lower oncotic pressure, supplementing this limited albumin response by upregulating the production of other proteins such as transferrin (also up by ~20%). Whether the body has the capacity to upregulate FcRn expression and thereby to respond to a hypoalbuminemic situation is not known.

We can imagine that the course of evolution has yielded a recycling mechanism for albumin and IgG, the two most abundant plasma proteins of the body, to supplement the capacity for biosynthesis. The magnitude of this effect is large, as illustrated by Fig. 4, which shows fluxes of albumin and IgG and indicates FcRn-dependent homeostasis of both proteins in the mouse at physiological conditions. It should be noted that the comparison is based on quantitative data for albumin turnover (using biosynthetically labeled albumin from this study) but only semiquantitative IgG data (using radioiodinated IgG decay) because our mouse strains maintain low plasma concentrations for IgG (1.8 mg/ml for WT and 0.18 mg/ml for KO mice) rendering biosynthetic rates for IgG unobtainable. At the physiological condition where the Css is maintained by homeostasis, the Rp equals the catabolic rate. It should be noted that while a much higher fraction of moles of IgG than albumin are recycled from degradation (7/9 rather than 1/2), the molar recycling ratio of albumin moles recycled to IgG moles recycled is astonishingly high, ~35:1 under physiological condition. When the IgG concentration in the mouse is hypothetically brought to the human equivalent of 10 mg/ml (66.7 μM), the molar recycling ratio (albumin/IgG) is still high, 6:1. This efficient FcRn-mediated recycling likely provides important survival value because of the essential nature of both ligands, albumin being the major transporter, colloid, and buffer of plasma, whereas IgG is the major protective

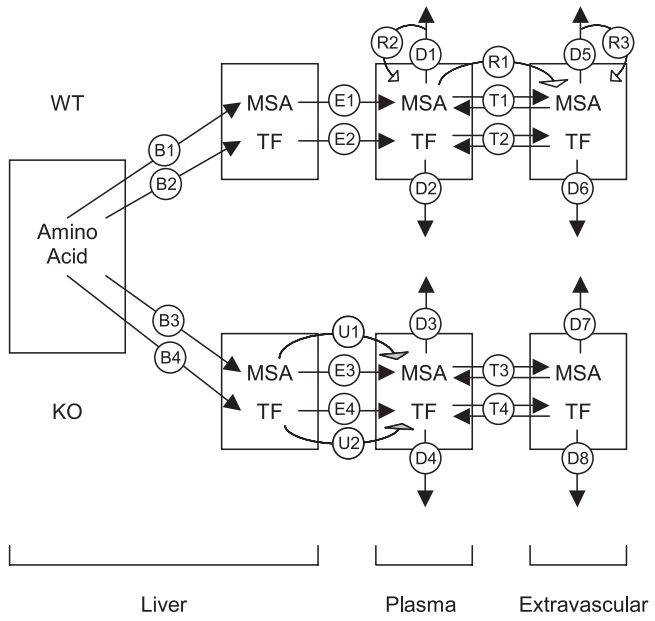

Fig. 5. Turnover model for albumin and transferrin and the role of FcRn. Whole body turnover of albumin (MSA) and transferrin (TF) in the presence and absence of FcRn (see DISCUSSION) is shown. Boxes indicate individual pools of amino acids or proteins in the liver, plasma compartment, and extravascular compartment.

antibody. Moreover, FcRn-mediated recycling of these abundant proteins may be bioenergetically more economical than the cost of additional protein synthesis and thus might provide a selective advantage in situations of food shortage.

On the basis of the data and our interpretation above, we propose a new albumin turnover model and indicate processes where FcRn is involved (Fig. 5). The model describes the homeostasis of albumin and transferrin in the presence of FcRn and indicates how a body reacts in response to hypoalbuminemia induced by the absence of FcRn. Previously, we had found that transferrin catabolism was not significantly different between the two strains based on the identical transferrin half-lives after intravenous administration of radioiodinated transferrin [36.7 ± 2.2 h (n = 15) and 36.9 ± 3.9 h (n = 15)] for WT and KO mice, respectively (unpublished data). Thus it would appear that both WT and KO mice would handle transferrin identically. Transferrin receptor-mediated transferrin recycling was therefore not indicated in this figure. Albumin and transferrin biosynthesized in hepatocytes (B1–B4) from amino acid pools of WT and KO mice are exported or secreted by exocytosis into the plasma compartment (E1–E4). Production or exocytosis (E1–E4) was measured in the current study. Because FcRn is responsible for albumin recycling (8) and was reported to be expressed in rat hepatocytes (5), we postulated that hepatocyte FcRn might salvage albumin from a "presecretory" degradation pathway. However, our data suggest that presecretory degradation and recycling are unlikely to occur in the liver. Therefore, the rate of biosynthesis appears to equal the rate of production with or without FcRn. The role of hepatic FcRn remains an interesting question. Albumin and transferrin in the plasma compartment are transported to and distribute reversibly in the extravascular compartment (T1–T4). FcRn is believed to participate in albumin transport from the plasma compartment to the extravascular compartment (R1; C. L. Bronson, unpublished data). Both compartments are proposed as potential sites for protein degradation (D1–D8), although early kinetic calculations indicated that degradation was limited to the plasma compartment in the normal animal, most likely to the endothelium (17). Likewise, both compartments are potential sites for FcRn-mediated recycling to protect albumin from cell-mediated degradation (R2–R3) because most cells of the body are endocytic and are believed to express FcRn. However, if no albumin degradation occurs in the extravascular compartment, then the cells of this compartment must be very efficient at FcRn recycling. Although it is not known per se how much the biosynthesis of albumin and transferrin is upregulated in KO mice (probably in response to a lower oncotic pressure caused by a lower plasma albumin concentration in the absence of FcRn), the existence of upregulation for the production of both albumin (U1) and transferrin (U2) contributes to the greater production rates of both proteins (~20%) in KO compared with WT mice. Our model in Fig. 5 further states that albumin only is recycled in the presence of FcRn, whereas in the absence of FcRn, the production of both albumin and transferrin is upregulated, resulting in a lower albumin and a higher transferrin concentration.

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