Effects of Controlled Feeding on Hepatic and Adrenal Cholesterol Metabolism in Mice: Post-print

Authors: Qian Hongliang, Pan Zhiqiang, Wang Xiaomin, Lu Tao, Fang Zhaoqin

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Abstract

This experiment aimed to investigate the effects of controlled feeding on cholesterol metabolism in the liver and adrenal glands of mice. Male ICR mice were used as experimental subjects. After 3 days of acclimation and reaching a body weight of 24-25 g, they were randomly divided into 5 groups (n=6 per group): normal control group (ad libitum feeding), 4.0 g feeding group [feed intake: 4.0 g/(mouse · day); restriction ratio: 88% of normal intake], 3.0 g feeding group [feed intake: 3.0 g/(mouse · day); restriction ratio: 66% of normal intake], 2.0 g feeding group [feed intake: 2.0 g/(mouse · day); restriction ratio: 44% of normal intake], and 1.5 g feeding group [feed intake: 1.5 g/(mouse · day); restriction ratio: 22% of normal intake]. All groups had free access to water. After 14 days of feeding control, the mice were sacrificed. Heart, liver, kidney, spleen, thymus, and testis were collected and weighed to calculate organ indices. Liver and adrenal tissues were collected for total RNA and total protein extraction. Real-time quantitative PCR was used to detect the relative mRNA expression levels of cholesterol metabolism-related genes, Western blot was used to detect the relative protein expression level of steroidogenic acute regulatory protein (StAR), and ELISA was used to detect serum corticosterone content. The results showed: 1) Compared with the normal control group, mice in all controlled feeding groups exhibited varying degrees of weight loss, and the heart, liver, kidney, spleen, and thymus showed varying degrees of atrophy. 2) Compared with the normal control group, the expression of hepatic low-density lipoprotein receptor (Ldlr), class B scavenger receptor 1 (Scarb1), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Hmgcr), hormone-sensitive lipase (Lipe), apolipoprotein E (Apoe), ATP-binding cassette transporter G5 (Abcg5), ATP-binding cassette transporter G8 (Abcg8), cytochrome P450 family 7 subfamily A member 1 (Cyp7a1), sterol regulatory element-binding protein cleavage-activating protein (Scap), liver X receptor beta (Nr1h2), peroxi-

some proliferator-activated receptor alpha (Ppara), and peroxisome proliferator-activated receptor gamma (Pparg) genes were significantly upregulated in the 3.0 g, 2.0 g, and 1.5 g feeding groups ($P<0.05$ or $P<0.01$), while the expression of insulin-induced gene 2 (Insig2) was significantly downregulated ($P<0.05$). 3) Compared with the normal control group, the expression of adrenal cytochrome P450 family 11 subfamily A member 1 (Cyp11a1) and cytochrome P450 family 21 subfamily A member 1 (Cyp21a1) genes were significantly upregulated in the 2.0 g and 1.5 g feeding groups ($P<0.05$). The expression of adrenal StAR and cytochrome P450 family 11 subfamily B member 1 (Cyp11b1) genes were also significantly upregulated in the 1.5 g feeding group ($P<0.05$), while the expression of cytochrome P450 family 11 subfamily B member 2 (Cyp11b2) gene was significantly downregulated in the 3.0 g, 2.0 g, and 1.5 g feeding groups ($P<0.05$). Additionally, compared with the normal control group, the expression of adrenal StAR protein was enhanced in all controlled feeding groups and increased with decreasing feed intake. These results indicate that strict feeding control affects the immune organs spleen and thymus in mice, while also severely impacting the metabolic organ liver, thereby influencing cholesterol metabolism and transformation in the liver and adrenal glands.

Full Text

Effects of Dietary Restriction on Cholesterol Metabolism in the Liver and Adrenal Gland of Mice

QIAN Hongliang, PAN Zhiqiang*, WANG Xiaomin, LU Tao, FANG Zhaoqin
(Basic Medical School, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China)

Abstract

This study investigated the effects of dietary restriction on cholesterol metabolism in the liver and adrenal gland of mice. Male ICR mice were acclimated for 3 days and, upon reaching 24–25 g body weight, randomly divided into five groups (n=6 each): a normal control group (ad libitum feeding), a 4.0 g feeding group [feed intake: 4.0 g/(mouse · day); restriction level: 88% of normal intake], a 3.0 g feeding group [feed intake: 3.0 g/(mouse · day); restriction level: 66% of normal intake], a 2.0 g feeding group [feed intake: 2.0 g/(mouse · day); restriction level: 44% of normal intake], and a 1.5 g feeding group [feed intake: 1.5 g/(mouse · day); restriction level: 22% of normal intake]. All groups had free access to water. After 14 days of dietary restriction, mice were euthanized. The heart, liver, kidneys, spleen, thymus, and testes were harvested, weighed, and organ indices calculated. Total RNA and protein were extracted from liver and adrenal tissues. Real-time quantitative PCR was used to measure relative mRNA expression of cholesterol metabolism-related genes, Western blot was performed to determine relative expression of the

steroidogenic acute regulatory protein (StAR), and ELISA was used to assay serum corticosterone levels.

The results showed: (1) Compared with the normal control group, all dietary restriction groups exhibited varying degrees of weight loss, with concurrent atrophy of the heart, liver, kidneys, spleen, and thymus. (2) In the 3.0 g, 2.0 g, and 1.5 g feeding groups, hepatic expression of low-density lipoprotein receptor (Ldlr), scavenger receptor class B type 1 (Scarb1), 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Hmgcr), hormone-sensitive lipase (Lipe), apolipoprotein E (Apoe), ATP-binding cassette transporter G5 (Abcg5), ATP-binding cassette transporter G8 (Abcg8), cytochrome P450 family 7 subfamily A member 1 (Cyp7a1), SREBP cleavage-activating protein (Scap), liver X receptor (Nr1h2), peroxisome proliferator-activated receptor (Ppara), and peroxisome proliferator-activated receptor (Pparg) were significantly upregulated ($P<0.05$ or $P<0.01$), whereas insulin-induced gene 2 (Insig2) expression was significantly downregulated ($P<0.05$). (3) In the 2.0 g and 1.5 g feeding groups, adrenal expression of cytochrome P450 family 11 subfamily A member 1 (Cyp11a1) and cytochrome P450 family 21 subfamily A member 1 (Cyp21a1) were significantly upregulated ($P<0.05$). In the 1.5 g feeding group, adrenal StAR and cytochrome P450 family 11 subfamily B member 1 (Cyp11b1) expression were also significantly upregulated ($P<0.05$), while cytochrome P450 family 11 subfamily B member 2 (Cyp11b2) expression was significantly downregulated in the 3.0 g, 2.0 g, and 1.5 g groups ($P<0.05$). Additionally, adrenal StAR protein expression was enhanced in all dietary restriction groups in a restriction-dependent manner. These findings demonstrate that severe dietary restriction profoundly affects immune organs (spleen and thymus) and metabolic organs (liver), consequently altering cholesterol metabolism and conversion in both the liver and adrenal gland.

Keywords: dietary restriction; mice; liver; adrenal gland; cholesterol metabolism

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Introduction

With economic development and improved living standards, dietary abundance has become commonplace. However, excessive food intake predisposes individuals to metabolic diseases, while insufficient intake also disrupts normal metabolism. Over two millennia ago, the *Neijing* emphasized that "diet should be regulated and flavors harmonized," recognizing that both inadequate and excessive consumption harm health. Numerous studies have established clear relationships between diet and chronic diseases such as obesity, diabetes, and hypertension [1-3], prompting many health-conscious individuals to restrict daily food intake, with obese individuals often imposing severe caloric limitation for weight loss. However, few reports have addressed potential adverse effects of dietary restriction.

Chronic undernutrition leads to nutritional imbalance, emaciation, and consumptive conditions. Diseases such as esophageal cancer, gastric cancer, and anorexia nervosa, characterized by inadequate dietary intake, cause metabolic abnormalities that manifest in Traditional Chinese Medicine (TCM) as qi deficiency, spleen-stomach weakness, or even dual deficiency of yin and yang [4]. Notably, TCM patterns of qi deficiency/spleen-stomach weakness closely relate to hepatic function, while kidney deficiency/dual yin-yang deficiency patterns associate with adrenal function. Both the liver and adrenal gland share a crucial biochemical characteristic as primary sites of cholesterol metabolism. Cholesterol is metabolized to bile acids in the liver, facilitating lipid digestion and absorption while preventing cholesterol precipitation and gallstone formation —processes that illuminate TCM concepts of spleen-stomach function. In the adrenal gland, cholesterol is converted to steroid hormones that mediate stress responses, reflecting aspects of TCM kidney function. Therefore, investigating the metabolic consequences of insufficient food intake may elucidate the material basis of TCM spleen-kidney deficiency patterns, particularly identifying which organs are most severely affected by substantial dietary restriction.

This study employed mice as experimental subjects, designing graded feeding regimens to identify critical thresholds of metabolic disruption and organ impact intensity, while exploring the underlying mechanisms of altered cholesterol metabolism following dietary restriction.

Materials and Methods

1.1.1 Experimental Animals and Diet Thirty male ICR mice (SPF grade, 6–8 weeks old, initial body weight 18–20 g) were purchased from Shanghai SIPPR-BK Laboratory Animal Co., Ltd. and housed in the SPF facility of Shanghai University of Traditional Chinese Medicine (Animal License No.: SCXK(Hu)2013-0016; Animal Certificate No.: 2008001672050). After 3 days of acclimation, mice were randomly assigned to experimental groups.

The basal diet (transgenic mouse formula, Cat. No. 1010011) was purchased from Jiangsu Synergetic Pharmaceutical & Biological Engineering Co., Ltd. Its composition and nutritional levels are presented in .

1.1.2 Reagents and Kits RIPA lysis buffer and enhanced chemiluminescence (ECL) kits were obtained from Beyotime Biotechnology. Rabbit polyclonal anti-steroidogenic acute regulatory protein (StAR) antibody was purchased from Abcam, and mouse anti- -actin antibody from Sigma-Aldrich. Trizol reagent was from Invitrogen. PrimeScript® RT Reagent Kit and SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) were from TaKaRa. Primers were designed using Primer3 (v.0.4.0) online software and synthesized by Life Technologies. Mouse corticosterone ELISA kit was from Cayman Chemical (USA).

1.1.3 Experimental Equipment Equipment included an Eco-Illumina real-time PCR system (Illumina, USA), Elx800 microplate reader (BioTek), and Eppendorf 5417R centrifuge.

1.2.1 Experimental Design Following 3 days of acclimation and upon reaching 24–25 g body weight, mice were randomly divided into five groups (n=6 each): normal control (ad libitum feeding), 4.0 g feeding [4.0 g/(mouse · day); 88% of normal intake], 3.0 g feeding [3.0 g/(mouse · day); 66% of normal intake], 2.0 g feeding [2.0 g/(mouse · day); 44% of normal intake], and 1.5 g feeding [1.5 g/(mouse · day); 22% of normal intake]. The normal control group received 100 g diet daily; remaining food was weighed after 24 h and replenished to 100 g. The four restriction groups received precisely measured daily rations (24, 18, 12, and 9 g for group cages, respectively), which were completely consumed within 24 h before replenishment. This feeding protocol continued for 14 days. Body weight was recorded every other day, and general condition was monitored.

1.2.2 Sample Collection On day 15, mice were euthanized. Samples were collected as follows: (1) Blood was collected via enucleation, serum was separated and stored at -20 °C for corticosterone measurement; (2) Heart, thymus, liver, spleen, kidneys (both), and testes (both) were harvested, weighed (in grams), and organ indices calculated as organ weight (g) to body weight (g) ratio; (3) Adrenal glands were collected for total RNA extraction to measure relative mRNA expression of -actin, StAR, Cyp11a1, Cyp21a1, Cyp11b1, and Cyp11b2; (4) Adrenal total protein was extracted for StAR protein quantification; (5) Liver tissue was collected for total RNA extraction to measure relative mRNA expression of cholesterol metabolism genes including -actin, Apoa1, Apoe, Abca1, Abcg1, Cyp7a1, Hmgcr, Ldlr, Nr1h3 (LXR), Nr1h2 (LXR), Ppara (PPAR), Pparg (PPAR), Scarb1 (SR-B1), Srebf1 (SREBP1), Srebf2 (SREBP2), Hmgcs1, Acat1, Lipe, Abcg5, Abcg8, Scap, Insig1, and Insig2.

1.2.3 Real-Time Quantitative PCR Primers were designed using Primer3 (v.0.4.0) and synthesized by Life Technologies (Shanghai). Total RNA from liver and adrenal tissues was extracted using Trizol reagent according to the manufacturer's protocol. Reverse transcription was performed in a 20 L reaction (37 °C for 15 min, 85 °C for 5 s, 4 °C). PCR amplification was conducted in a 20 L volume with the following program: 95 °C for 3 min, followed by 40 cycles of 95 °C for 30 s and 60 °C for 30 s. Relative mRNA expression was calculated using the 2^(-ΔΔCt) method, with the normal control group as calibrator and -actin as internal reference: ΔCt = Ct_target - Ct_reference; ΔΔCt = ΔCt_restriction - ΔCt_control; Relative expression = 2^(-ΔΔCt). Primer sequences are listed in (liver genes) and (adrenal genes).

1.2.4 Western Blot Analysis for StAR Protein Tissues were lysed with RIPA buffer. Total protein was collected, quantified, and denatured. Samples were subjected to SDS-PAGE, transferred to membranes, blocked, incubated

with primary antibodies (-actin at 1:20,000; StAR at 1:2,000), washed, incubated with secondary antibodies, washed again, and visualized. -actin served as internal reference for relative StAR protein quantification.

1.2.5 ELISA for Serum Corticosterone Serum samples were assayed undiluted using the mouse corticosterone ELISA kit according to the manufacturer' s instructions.

1.3 Statistical Analysis GraphPad Prism 5.0 was used for graphing and statistical analysis. Body weight data were analyzed by repeated measures, comparing each restriction group to the normal control. Organ weights and indices were analyzed by one-way ANOVA. $P<0.05$ was considered statistically significant.

Results

2.1 Dynamic Changes in Body Weight Normal control mice showed gradual weight gain, reaching 30 g by day 15. The 4.0 g feeding group exhibited slight but non-significant weight reduction compared with controls ($P>0.05$). In contrast, the 3.0 g, 2.0 g, and 1.5 g feeding groups showed significant weight loss from day 5 onward ($P<0.05$), with the degree of loss correlating with restriction severity. These results indicate that feed intake 3.0 g/(mouse · day) induces weight loss in mice.

Data points marked with "*" indicate significant difference from normal control ($P<0.05$).

[Figure 1: see original paper] Comparison of body weight changes among groups

2.2.1 Heart Weight and Cardiac Index As shown in , heart weight decreased significantly in all restriction groups compared with controls ($P<0.05$, $P<0.01$, or $P<0.001$), with progressive reduction as feed intake decreased. However, cardiac index remained unchanged across groups ($P>0.05$), indicating that heart weight loss paralleled overall body weight reduction.

2.2.2 Liver Weight and Hepatic Index Liver weight decreased significantly in all restriction groups ($P<0.05$), most markedly in groups receiving 3.0 g/(mouse · day) ($P<0.001$), with concurrent significant reduction in hepatic index ($P<0.001$). This suggests that the liver, as a major metabolic organ, exhibited the most pronounced atrophy following dietary restriction.

2.2.3 Kidney Weight and Renal Index Kidney weight decreased significantly in all restriction groups ($P<0.05$ or $P<0.001$), particularly in those receiving 3.0 g/(mouse · day) ($P<0.001$). Renal index also decreased significantly in the 3.0 g and 2.0 g groups ($P<0.05$), indicating notable renal atrophy.

2.2.4 Spleen Weight and Splenic Index Spleen weight decreased significantly in groups receiving 3.0 g/(mouse · day) ($P<0.001$), with splenic index also reduced, most notably in the 2.0 g group ($P<0.05$). This demonstrates that spleen atrophy was more severe than overall body weight loss when feed intake fell below 3.0 g/(mouse · day).

2.2.5 Thymus Weight and Thymic Index Thymus weight decreased significantly in groups receiving 3.0 g/(mouse · day) ($P<0.01$ or $P<0.001$), with thymic index reduced, particularly in the 1.5 g group ($P<0.05$). These findings indicate that severe dietary restriction causes thymic atrophy and compromised immune function.

2.2.6 Testis Weight and Testicular Index Testis weight showed no significant change across groups ($P>0.05$). However, testicular index increased significantly in the 2.0 g and 1.5 g groups ($P<0.01$ or $P<0.001$), suggesting that testes did not atrophy relative to body weight loss and may be relatively protected from dietary restriction.

In summary, severe dietary restriction most profoundly affected immune organs (spleen and thymus), followed by metabolic organs (liver and kidneys), with minimal impact on the testes.

Changes in organ weights and organ indices of mice

2.3.1 Cholesterol Uptake As shown in , Ldlr and Scarb1 expression were significantly upregulated in groups receiving 3.0 g/(mouse · day) ($P<0.05$). Since low-density lipoprotein receptor (LDLR, encoded by Ldlr) and scavenger receptor class B type 1 (SR-B1, encoded by Scarb1) mediate hepatocellular cholesterol uptake, these results suggest that reduced dietary cholesterol intake triggered compensatory upregulation of these receptors to enhance hepatic cholesterol uptake from circulation.

2.3.2 Cholesterol Synthesis shows that Hmgcr expression was significantly upregulated in groups receiving 3.0 g/(mouse · day) ($P<0.05$ or $P<0.01$), while Hmgcs1 expression remained unchanged ($P>0.05$). As HMG-CoA reductase (HMGCR, encoded by Hmgcr) catalyzes the rate-limiting step in de novo cholesterol synthesis, these findings indicate that dietary restriction stimulated hepatic cholesterol synthesis through Hmgcr upregulation.

2.3.3 Cholesterol Storage demonstrates that Lipe expression was significantly upregulated in the 3.0 g and 2.0 g groups ($P<0.05$), whereas Acat1 expression, which promotes esterification, remained unchanged ($P>0.05$). This suggests that dietary restriction enhanced hydrolysis of esterified cholesterol from lipid droplets to provide free cholesterol for cellular needs.

2.3.4 Cholesterol Efflux shows that Apoe, Abcg5, and Abcg8 expression were unchanged in the 4.0 g group (P>0.05) but significantly upregulated in groups receiving 3.0 g/(mouse·day) (P<0.05). In contrast, Apoa1, Abca1, and Abcg1 expression remained unchanged across all restriction groups (P>0.05). These results indicate that severe dietary restriction increased hepatic cholesterol efflux, reflecting heightened metabolic activity.

2.3.5 Cholesterol Metabolic Conversion reveals that Cyp7a1 and Scap expression were significantly upregulated in all restriction groups except the 4.0 g group for Cyp7a1 (P<0.05 or P<0.01). This suggests that dietary restriction accelerated hepatic conversion of cholesterol to bile acids.

2.3.6 Cholesterol Homeostasis shows that Nr1h2, Ppara, and Pparg expression were significantly upregulated in groups receiving 3.0 g/(mouse · day) (P<0.05), while Insig2 expression was significantly downregulated (P<0.05). Expression of Nr1h3, Srebf1, Srebf2, and Insig1 remained unchanged (P>0.05). These findings indicate that dietary restriction activated key regulators of cholesterol homeostasis including liver X receptor (LXR , encoded by Nr1h2), peroxisome proliferator-activated receptor (PPAR , encoded by Ppara), and PPAR (encoded by Pparg).

Collectively, these results demonstrate that substantial dietary restriction, particularly severe caloric limitation, significantly alters expression of genes involved in hepatic cholesterol synthesis and metabolism, while hepatic cells actively maintain cholesterol homeostasis through compensatory mechanisms.

2.4 Key Gene Expression Changes in Adrenal Cholesterol-to-Corticosteroid Conversion shows that Cyp11a1 and Cyp21a1 expression were significantly upregulated in the 2.0 g and 1.5 g groups (P<0.05). In the 1.5 g group, StAR and Cyp11b1 expression were also significantly upregulated (P<0.05), while Cyp11b2 expression was significantly downregulated in the 3.0 g, 2.0 g, and 1.5 g groups (P<0.05). Since StAR and CYP11A1 are rate-limiting enzymes for cholesterol conversion to pregnenolone, and CYP21A1 and CYP11B1 direct corticosteroid synthesis, these results suggest that severe dietary restriction enhanced adrenal cholesterol conversion to corticosteroids to adapt to metabolic stress. Conversely, downregulation of Cyp11b2, which encodes aldosterone synthase, indicates impaired mineralocorticoid synthesis and potential electrolyte imbalance.

2.5 Adrenal StAR Protein Expression [Figure 2: see original paper] demonstrates that adrenal StAR protein expression was enhanced in all restriction groups in a restriction-dependent manner, increasing as feed intake decreased. This compensatory upregulation likely facilitates cholesterol transport for steroidogenesis.

2.6 Effects of Dietary Restriction on Serum Corticosterone [Figure 3: see original paper] shows that serum corticosterone levels decreased slightly in the 4.0 g and 3.0 g groups but remained essentially unchanged in the 1.5 g group, with no significant differences compared with controls ($P>0.05$).

Discussion

3.1 Effects of Dietary Restriction on Body Weight and Major Organs Food represents the most fundamental requirement for mammals. Chronic undernutrition inevitably disrupts metabolism, leading to emaciation and cachexia. The *Neijing* described the complex transformation and distribution of dietary nutrients: "Food and drink enter the stomach, where essential qi overflows and ascends to the spleen; spleen qi disperses the essence, which ascends to the lungs, regulates the waterways, and descends to the bladder. Thus, water and essence are distributed throughout the body, flowing through the five meridians in harmony with the four seasons and the yin-yang of the five viscera" [5]. This ancient text recognized that insufficient intake impairs normal visceral function. Clinically, patients who restrict diet due to anorexia or inability to eat develop emaciation and manifest metabolic abnormalities.

From a therapeutic perspective, increasing food intake can correct metabolic dysfunction. However, researchers must understand what metabolic abnormalities occur with reduced intake and what restriction level minimally impacts metabolism. Moderate dietary restriction within certain limits confers beneficial effects on multiple physiological functions. Chen et al. [6] reported that mechanisms underlying metabolic improvement during caloric restriction involve several pathways: (1) increased ketone bodies, where elevated -hydroxybutyrate enhances antioxidant stress factors FOXO3A and MT2 to protect against oxidative damage; (2) reduced leptin levels and improved leptin resistance; and (3) decreased insulin-like growth factor 1 (IGF-1), with downregulated IGF-1/insulin signaling linked to anti-aging, anti-tumor, and lifespan extension effects.

Our results indicate that for 25 g male ICR mice, normal ad libitum intake is typically 4–5 g/day (approximately 18% of body weight). At 88% of normal intake [4.0 g/(mouse · day), ~16% of body weight], mice showed mild weight and organ reduction. At 66% of normal intake [3.0 g/(mouse · day), ~12% of body weight], significant atrophy occurred in body weight, heart, liver, kidneys, spleen, and thymus. At 44% [2.0 g/(mouse · day), ~8% of body weight] and 22% [1.5 g/(mouse · day), ~6% of body weight] of normal intake, atrophy became more pronounced. These findings suggest that two-thirds of normal intake represents a critical threshold; beyond this level, the spleen, thymus, and liver are primarily affected, followed by heart and kidneys, while testes remain relatively unaffected. Xie [7] found that high nutrition enhanced reproductive capacity in male Drosophila by increasing sperm bundle number and testis volume, whereas dietary restriction had opposite effects. Both conditions accelerated reproduc-

tive system aging at advanced age, with restriction extending lifespan by reducing reproductive capacity while high nutrition shortened lifespan by enhancing it, suggesting a trade-off between survival and reproduction. In our study, restricted mice showed higher daily activity, and reproductive organ weight did not decline, suggesting reproductive function remains preserved during dietary restriction.

3.2 Effects of Dietary Restriction on Hepatic Cholesterol Metabolism
Cholesterol, widely distributed in animals, plays vital biological roles in growth and reproduction. Both deficiency and excess impair normal physiological function. Our results show that feeding 66% of normal intake [1.5–3.0 g/(mouse · day)] significantly upregulated hepatic expression of Ldlr, Scarb1, Hmgcr, Lipe, Apoe, Abcg5, Abcg8, Cyp7a1, Scap, Nr1h2, Ppara, and Pparg, while downregulating Insig2. This indicates that restriction beyond two-thirds of normal intake disrupts expression of genes governing hepatic cholesterol uptake, synthesis, storage, efflux, metabolism, and homeostatic regulation.

LDLR mediates cellular LDL uptake and metabolism, clearing intermediate-density lipoproteins and reducing LDL generation to maintain plasma LDL stability; LDLR dysfunction causes lipid metabolism disorders [8]. SR-B1, the only confirmed HDL receptor and primarily expressed in liver, binds HDL, native LDL, oxidized LDL, and acetylated LDL, participating in reverse cholesterol transport to remove excess peripheral cholesterol and prevent atherosclerosis [9]. HMGCR catalyzes the rate-limiting step in de novo cholesterol synthesis and represents a key regulatory enzyme [10]. Our findings suggest that severe dietary restriction activates hepatic processes for cholesterol uptake, synthesis, and reverse transport, reducing circulating cholesterol levels.

Furthermore, cholesterol 7 -hydroxylase (encoded by Cyp7a1) is the rate-limiting enzyme for bile acid synthesis and is regulated by fibroblast growth factor 15 (FGF15) [11]. ATP-binding cassette (ABC) transporters ABCG5 and ABCG8 (encoded by Abcg5 and Abcg8) inhibit intestinal cholesterol absorption and promote biliary cholesterol secretion [12-13]. ABCA1 and ABCG1 participate in reverse cholesterol transport [14]. Nuclear receptor superfamily members including liver X receptors (LXR encoded by Nr1h3, LXR encoded by Nr1h2) and peroxisome proliferator-activated receptors (PPAR encoded by Ppara, PPAR encoded by Pparg) regulate cholesterol metabolism by modulating Ldlr and Scarb1 expression. PPAR is the target of fibrates, while PPAR regulates glucose and lipid metabolism genes and represents an important target for type 2 diabetes therapy. Thus, LXR , LXR , PPAR , and PPAR are considered key regulators of cholesterol homeostasis. Jakulj et al. [12] demonstrated that severe caloric restriction upregulates Abcg5 and Abcg8 to inhibit intestinal cholesterol absorption, upregulates Cyp7a1 to accelerate bile acid synthesis, and activates Nr1h2, Ppara, and Pparg to regulate cholesterol homeostasis.

3.3 Effects of Dietary Restriction on Adrenal Cholesterol Conversion

The adrenal gland converts cholesterol to steroid hormones, particularly glucocorticoids and mineralocorticoids, which regulate critical metabolic processes. Steroidogenic enzymes play essential biochemical roles [17]. Our study found that feeding 44% of normal intake [1.5–2.0 g/(mouse·day)] significantly upregulated adrenal Cyp11a1 and Cyp21a1 expression, indicating active conversion of cholesterol to corticosteroids during severe restriction. At 22% of normal intake [1.5 g/(mouse · day)], StAR gene and protein expression and Cyp11b1 expression were significantly upregulated, while Cyp11b2 expression was downregulated. This suggests that severe restriction enhanced cholesterol transport into mitochondria for steroidogenesis, with increased corticosterone synthesis but suppressed aldosterone production. Notably, serum corticosterone decreased only slightly at 88% and 66% of normal intake, without correlating with gene and protein expression patterns. We speculate that corticosterone synthesis may be actively compensated at the transcriptional level, yet actual secretion remains inadequate, possibly due to accelerated corticosterone metabolism and urinary excretion.

Conclusions

1. Dietary restriction exceeding one-third of normal intake affects body weight and organ mass in ICR mice, with the spleen, thymus, and liver most severely affected, followed by heart and kidneys, while testes remain minimally impacted. This suggests that severe dietary restriction primarily damages immune and metabolic organs, while reproductive organs exhibit robust self-protection to preserve reproductive function.
2. Following substantial dietary restriction, key genes involved in hepatic cholesterol uptake, synthesis, and metabolism are upregulated, representing a compensatory mechanism to maintain hepatocellular cholesterol homeostasis.
3. Severe dietary restriction activates adrenal cortical cholesterol-to-steroid hormone conversion, enhancing corticosteroid synthesis to adapt to metabolic stress.

References

[1] JIANG Zhongli, ZHANG Qin, HE Danjun, et al. Analysis of dietary behavior in obese and diabetic patients[J]. Chinese Journal of Physical Medicine and Rehabilitation, 2000, 22(6): 343–345.

[2] LIU Lisong, HUA Qi, PANG Beilei. Dietary structure and cardiovascular risk factors in patients with essential hypertension[J]. Chinese Journal of Hypertension, 2008, 16(3): 225–229.

[3] CHEN Mingdao, LI Rongying. Different viewpoints on dietary therapy for diabetes[J]. Chinese Journal of Internal Medicine, 2005, 44(10): 791-793.

[4] ZHANG Junjie, LI Liang, KE Bin. Exploring the mechanism of spleen deficiency induced by dietary irregularities from the perspective of carnitine homeostasis[J]. Lishizhen Medicine and Materia Medica Research, 2016, 27(5): 1257-1259.

[5] WANG Qingqi. Selected Readings from Neijing[M]. Beijing: China Press of Traditional Chinese Medicine, 2007: 59-60.

[6] CHEN Guofang, LIU Chao. Effects of caloric restriction on metabolic diseases and related mechanisms[J]. Journal of Diagnostics Concepts & Practice, 2016, 15(4): 346-349.

[7] XIE Dejuan. Effects and mechanisms of dietary restriction and high nutrition on aging and reproduction in male Drosophila[D]. Master' s thesis. Xi' an: Shaanxi Normal University, 2008.

[8] ZELCER N, HONG C, BOYADJIAN R, et al. LXR regulates cholesterol uptake through Idol-dependent ubiquitination of the LDL receptor[J]. Science, 2009, 325(5936): 100-104.

[9] HEINECKE J W. Small HDL promotes cholesterol efflux by the ABCA1 pathway in macrophages: implications for HDL-targeted therapies[J]. Circulation Research, 2015, 116(7): 1101-1103.

[10] TALL A R, YVAN-CHARVET L, TERASAKA N, et al. HDL, ABC transporters, and cholesterol efflux: implications for the treatment of atherosclerosis[J]. Cell Metabolism, 2008, 7(5): 365-375.

[11] INAGAKI T, CHOI M, MOSCHETTA A, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis[J]. Cell Metabolism, 2005, 2(4): 217-225.

[12] JAKULJ L, VAN DIJK T H, DE BOER J F, et al. Transintestinal cholesterol transport is active in mice and humans and controls ezetimibe-induced fecal neutral sterol excretion[J]. Cell Metabolism, 2016, 24(6): 783-794.

[13] YUAN Min, WANG Min, FU Wenliang, et al. SAK-HV protein reduces cholesterol absorption by upregulating ABCG5/ABCG8 expression[J]. Journal of Medical Research, 2015, 44(7): 17-22.

[14] KENNEDY M A, BARRERA G C, NAKAMURA K, et al. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation[J]. Cell Metabolism, 2005, 1(2): 121-131.

[15] PEET D J, TURLEY S D, MA W Z, et al. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR [J]. Cell, 1998, 93(5): 693-704.

[16] HANKIR M K, SEYFRIED F, HINTSCHICH C A, et al. Gastric bypass surgery recruits a gut PPAR- -striatal pathway to reduce appetite in obese rats[J]. Cell Metabolism, 2017, 25(2): 335-344.

[17] MILLER W L, AUCHUS R J. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders[J]. Endocrine Reviews, 2011, 32(1): 81-151.

Note: Figure translations are in progress. See original paper for figures.