Table 2
Food consumption and body weight gain in Zucker obese rats fed graded levels of *Garcinia cambogia* for 92 days in G2 to G5 and 93 days in G1

		Group/HCA (mmol/kg)/wt%				
		G1/154/3.0	G2/102/2.0	G3/51/1.0	G4/10/0.2	G5 (control)/0/0.0
Food consumption	g/d	15.1 ± 1.1	14.8 ± 0.9	14.8 ± 1.0	14.9 ± 1.1	14.7 ± 1.2
HCA consumption[a]	mg/d	452.7	296.1	147.6	29.8	0.0
	mg/kg BW/d	1243.8	778.2	388.9	77.5	0.0
	mmol/d	2.30	1.51	0.75	0.15	0.00
	mmol/kg BW/d	6.33	3.96	1.98	0.39	0.00
Body weight gain	g/d	2.4 ± 0.3	2.5 ± 0.1	2.5 ± 0.2	2.5 ± 0.2	2.6 ± 0.2

HCA, (−)-hydroxycitric acid.
Each value is the mean ± S.D. $n = 6$.
[a] Average.

Table 3
Testis and epididymal fat pad weights, activities of ATP-citrate lyase in liver and epididymal fat pad, and concentrations of liver glycogen, plasma NEFA and serum leptin in Zucker obese rats fed graded levels of *Garcinia cambogia*

		Group/HCA (mmol/kg)				
		G1/154	G2/102	G3/51	G4/10	G5 (control)/0
Testis	g	0.85 ± 0.18a	0.88 ± 0.10a	1.98 ± 0.15b	1.83 ± 0.55b	1.97 ± 0.16b
Epididymal fat pad	g	9.67 ± 1.43a	12.73 ± 1.61b	12.17 ± 2.04b	13.65 ± 1.34b	13.22 ± 2.48b
	g/100 gBW	2.66 ± 0.31a	3.37 ± 0.57b	3.22 ± 0.59ab	3.55 ± 0.24b	3.50 ± 0.66b
ATP-citrate lyase						
Liver	nmol/mg protein/min	67.9 ± 14.0a	61.3 ± 11.8ab	46.9 ± 19.5bc	41.3 ± 9.1c	41.4 ± 17.5c
Epididymal fat pad	nmol/mg protein/min	5.48 ± 2.67a	8.62 ± 6.30a	8.33 ± 2.81a	8.48 ± 2.61a	9.27 ± 5.21a
Liver glycogen	mg/g liver	11.3 ± 4.2a	10.5 ± 4.0a	5.7 ± 2.4b	4.2 ± 2.6b	4.2 ± 3.0b
Plasma NEFA	mmol/l	0.47 ± 0.08a	0.69 ± 0.07bc	0.65 ± 0.07b	0.84 ± 0.24c	0.77 ± 0.12bc
Serum leptin	ng/ml	68.9 ± 30.0a	73.3 ± 23.0a	67.1 ± 22.3a	74.7 ± 25.5a	63.2 ± 29.0a

HCA, (−)-hydroxycitric acid; NEFA, non-esterified fatty acid.
Each value is the mean ± S.D. $n = 6$. Means in a row that are not followed by a common letter are different. Significance of differences between mean values was assessed by 1-way ANOVA coupled with Duncan's multiple-range test at the 5% level of significance.

shown). Epididymal fat pad weights (absolute and relative) were significantly lower in the highest HCA group (G1) than in the other groups.

3.3. ATP-citrate lyase activity and concentrations of liver glycogen, plasma NEFA and serum leptin

The activities of liver ATP-citrate lyase became lower as the dietary level of HCA decreased (Table 3), but there was no significant difference in the activities of the epididymal fat pad among any of the treatment groups, although that of the highest HCA group (G1) tended to be lower than those of the other groups. Liver glycogen concentrations became lower as the dietary level of HCA decreased, and those of the highest and second highest HCA groups (G1 and G2) were significantly higher than those of the other groups (G3 to G5). The plasma NEFA concentration was significantly lower in the highest HCA group (G1) and tended to become higher as the dietary level of HCA decreased, but the differences among groups G2 to G5 were not statistically significant. There was no significant difference in the serum leptin concentration among any of the treatment groups.

3.4. Histopathological examination

As marked atrophy of the testis was noticed at autopsy, histopathological examination of the testis, together with the liver and spleen, was performed by H.E. staining. Marked atrophy and degeneration of germ cells (Fig. 1C and D) were observed in the highest and second highest HCA groups (G1 and G2); the degeneration was dose-dependent and most marked in G1 (Table 4). However, abnormal findings were not seen morphologically in Sertoli cells and Leydig cells even in the highest and second highest HCA groups. No histopathological changes in the testis were observed in the other three groups, G3 to G5 (Table 4 and Fig. 1A and B). In the liver, fat accumulation as judged by fat vacuolation was observed in all treatment groups (Table 4), and the accumulation was conspicuous in both the highest HCA group (G1) and the control group (G5). No histopathological changes caused by *Garcinia cambogia* were observed in the spleen (data not shown).

3.5. Serum biochemical parameters

No obvious changes associated with tissue cell injury were observed in any of the treatment groups (Table 5). ALP activity was significantly lower and blood urea nitrogen (BUN) concentration was significantly higher in the highest HCA group (G1) compared with those in the control group (G5), respectively.

As shown in Table 6, there were no significant differences in the plasma concentrations of testosterone and

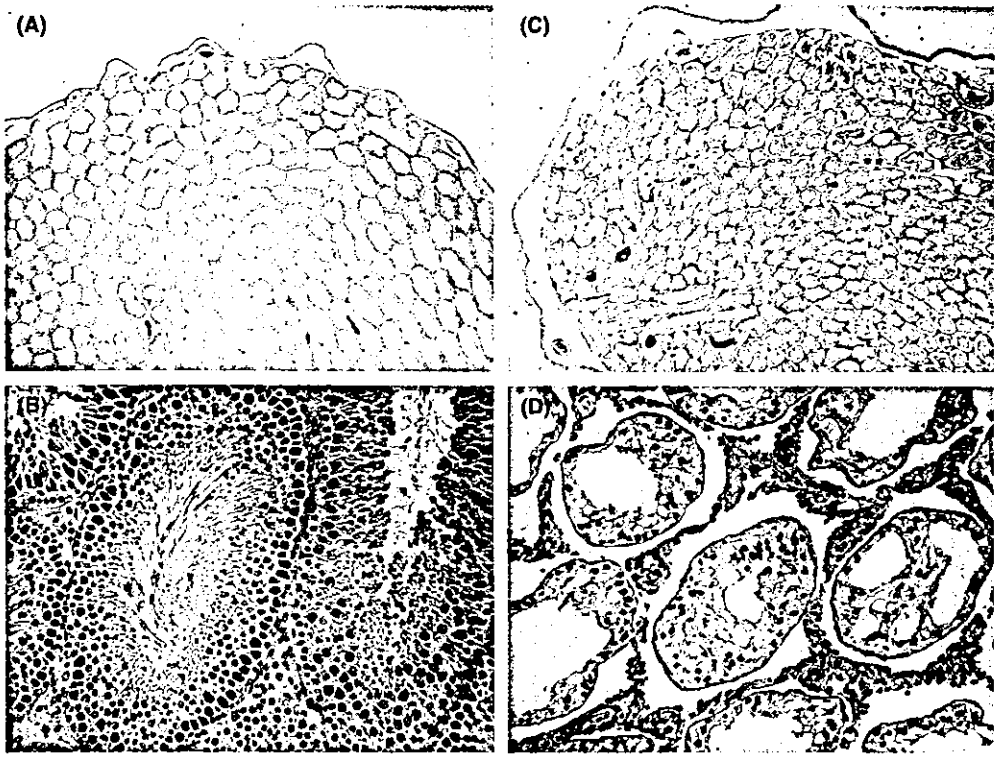

Fig. 1. Histopathological examination of the testis in Zucker obese rats fed graded levels of *Garcinia cambogia*. A: G5 (control) (H.E. stain, × 20), no significant changes; B: higher magnification of A (H.E. stain, × 200); C: G1 (H.E. stain, × 20), note marked atrophy (+++); D: higher magnification of C (H.E. stain, × 200), note marked degeneration of germ cells (+++). Abnormal findings were not seen morphologically in Sertoli cells and Leydig cells.

Table 4
Histopathological findings in testis and liver in Zucker obese rats fed graded levels of *Garcinia cambogia*

		Group/HCA (mmol/kg)/No. of animals examined				
		G1/154/6	G2/102/6	G3/51/6	G4/10/6	G5 (control)/0/6
Testis						
Germ cell degeneration	–	0	0	6	6	6
	+	0	2	0	0	0
	++	0	2	0	0	0
	+++	6	2	0	0	0
Atrophy	–	0	0	6	6	6
	+	0	0	0	0	0
	++	0	4	0	0	0
	+++	6	2	0	0	0
Liver						
Fat accumulation	–	0	0	0	0	0
	+	0	0	0	2	0
	++	0	2	2	1	0
	+++	6	4	4	3	6

HCA, (−)-hydroxycitric acid.
−: negative; +: mild; ++: moderate; +++: marked.

Table 5
Serum biochemical parameters in Zucker obese rats fed graded levels of *Garcinia cambogia*

		Group/HCA (mmol/kg)				
		G1/154	G2/102	G3/51	G4/10	G5 (control)/0
Total protein	g/l	64.2 ± 2.9a	61.8 ± 1.5ab	59.7 ± 1.4b	63.6 ± 2.9a	60.8 ± 4.1ab
Albumin	g/l	30.2 ± 1.9ac	30.3 ± 1.4ac	29.2 ± 1.7bc	31.8 ± 2.3a	29.7 ± 2.2ac
A/G		0.87 ± 0.08a	0.93 ± 0.05ac	0.97 ± 0.10ac	1.00 ± 0.10bc	0.97 ± 0.05ac
AST	μkat/l	3.6 ± 2.0a	3.5 ± 1.0a	4.9 ± 1.9a	3.4 ± 1.5a	3.6 ± 0.6a
ALT	μkat/l	4.1 ± 2.1a	4.5 ± 1.5a	7.1 ± 3.4a	3.9 ± 2.7a	4.6 ± 1.1a
γ-GTP	μkat/l	0.02 ± 0.00a	0.02 ± 0.00a	0.02 ± 0.00a	0.02 ± 0.00a	0.02 ± 0.00a
ALP	μkat/l	9.3 ± 1.3a	10.8 ± 1.3ac	12.4 ± 1.8bc	10.1 ± 3.2ac	12.0 ± 1.9bc
Creatinine	μmol/l	30.9 ± 4.8ab	29.5 ± 4.6ac	26.5 ± 0.0bc	33.6 ± 4.0a	29.5 ± 4.6ac
BUN	mmol/l	8.0 ± 2.0a	4.9 ± 1.1b	4.7 ± 0.5bc	3.4 ± 0.5c	4.0 ± 0.6bc
Total bilirubin	μmol/l	1.71 ± 0.00a	1.71 ± 0.00a	1.71 ± 0.00a	2.05 ± 0.76a	1.71 ± 0.00a

HCA, (−)-hydroxycitric acid; A/G, albumin/globulin; AST, L-aspartate:2-oxoglutarate aminotransferase; ALT, L-alanine:2-oxoglutarate aminotransferase; γ-GTP, γ-glutamyltranspeptidase; ALP, alkaline phosphatase; BUN, blood urea nitrogen. Each value is the mean ± S.D. $n = 6$. Means in a row that are not followed by a common letter are different.
Significance of differences between mean values was assessed by 1-way ANOVA coupled with Duncan's multiple-range test at the 5% level of significance.

Table 6
Concentrations of testosterone, LH, inhibin-B and FSH in plasma in Zucker obese rats fed graded levels of *Garcinia cambogia*

		Group/HCA (mmol/kg)				
		G1/154	G2/102	G3/51	G4/10	G5 (control)/0
Testosterone	ng/ml	0.19 ± 0.16a	0.20 ± 0.11a*	0.19 ± 0.09a*	0.15 ± 0.08a**	0.13 ± 0.03a
LH	ng/ml	3.85 ± 1.42a	2.32 ± 0.25a	5.22 ± 3.80a	4.92 ± 5.99a*	3.18 ± 0.98a
Inhibin-B	pg/ml	n.d. a	n.d. a	25.0 ± 15.8b	21.6 ± 3.9b*	26.5 ± 10.2b
FSH	ng/ml	37.62 ± 13.39a	31.35 ± 11.87a	9.95 ± 1.80b	9.66 ± 3.99b*	10.40 ± 2.13b

HCA, (−)-hydroxycitric acid; LH, luteinizing hormone; FSH, follicle-stimulating hormone. n.d., not detected. Each value is the mean ± S.D. $n = 6$. Means in a row that are not followed by a common letter are different.
Significance of differences between mean values was assessed by 1-way ANOVA coupled with Duncan's multiple-range test at the 5% level of significance.
* $n = 5$.
** $n = 4$.

LH among any of the treatment groups. The plasma concentrations of inhibin-B in the highest and second highest HCA groups (G1 and G2) were significantly lower and those of FSH in the same groups significantly higher than those of the other three groups (G3 to G5), but the concentrations between the latter three groups (G3 to G5) were not significantly different, respectively.

4. Discussion

The Zucker obese rat has been used extensively as a model of early-onset obesity. In addition to hyperphagia caused by leptin receptor missense mutation (Iida et al., 1996; Phillips et al., 1996) and hyperplastic–hypertrophic adipose depots (Greenwood et al., 1981), Zucker obese rats are characterized by hypercholesterolemia, hyperlipidemia, hyperleptinemia, hyperinsulinemia and insulin resistance as a recessive trait (Cleary et al., 1987; Shimomura et al., 1992), featuring similar to human obesity.

HCA-containing *Garcinia cambogia* has been shown to be active in suppressing appetite and body fat accumulation in experimental animals (Greenwood et al., 1981; Ishihara et al., 2000; Ohia et al., 2002; Rao and Sakaria, 1988; Sullivan and Triscari, 1977; Sullivan et al., 1974a; Vasselli et al., 1998). However, an usual level of HCA around 50 mmol/kg diet used in many previous studies (Chee et al., 1977; Greenwood et al., 1981; Rao and Sakaria, 1988; Sullivan and Triscari, 1977) was ineffective in suppressing body fat accumulation in developing Zucker obese (fa/fa) rats. This ineffectiveness may be due to the several important metabolic characteristics of Zucker obese rats, such as elevated adipose tissue lipoprotein lipase activity (Cleary et al., 1980; Gruen et al., 1978; Peinado-Onsurbe et al., 2001) and acyl-CoA synthetase activity (Shimomura et al., 1992), which contribute to increase lipogenesis. Thus, Zucker obese rats and other animal species with higher lipogenic properties appear to be insensitive to HCA treatment at the usual dietary levels. Therefore, in this study we administered higher levels of HCA in order to determine dose–response relationships in a long-term pair-feeding with a constant energy intake in developing Zucker obese rats.

As a result, a significant suppression of body fat accumulation was observed in epididymal adipose tissue in

the highest dietary level of HCA (Table 3). As ATP-citrate lyase activity in epididymal adipose tissue tended to be lower in the highest HCA group than in the other four groups (Table 3), high-dose HCA might suppress fatty acid synthesis, and thus suppress lipogenesis and epididymal fat accumulation, through the decreased activity of ATP-citrate lyase. This is not mediated by suppression of appetite and food intake because similar food intake with a constant energy and similar bodyweight gain were observed in all of the treatment groups (Table 2). This presumption may be supported by the observations in this study that the ATP-citrate lyase activity and glycogen concentration in the liver became higher and the plasma NEFA concentration became lower as the dietary level of HCA increased (Table 3). Zucker obese rats are hyperphagic, and thus the rats receiving lower dietary levels of HCA but pair-fed with the highest HCA group may experience hunger. Similar results have been obtained in Sprague–Dawley rats, where animals consuming a diet devoid of HCA but pair-fed with HCA-treated free-feeding rats had significantly lower activities of ATP-citrate lyase and fatty acid synthetase in the liver (Chee et al., 1977). The anorectic action of HCA in the present study is not mediated by leptin, because Zucker obese rats with a leptin receptor missense mutation were used, and thus, the serum leptin concentrations were not different among any of the treatment groups (Table 3).

The ineffectiveness of the lower levels of dietary HCA against fat accumulation may be explained by the metabolic characteristics unique to Zucker obese rats, such as elevated adipose tissue lipoprotein lipase activity (Cleary et al., 1980; Gruen et al., 1978; Peinado-Onsurbe et al., 2001), with high incorporation of circulating lipids, and also elevated adipose tissue acyl-CoA synthetase activity (Shimomura et al., 1992), with enhanced lipogenesis.

Therefore, it appears from the results obtained herein that obese people who are genetically predisposed to the alterations in lipid metabolism, characteristic of Zucker obese rats, would be unlikely to benefit practically from HCA derived from *Garcinia cambogia* in terms of suppression of body fat accumulation. Moreover, acetyl-CoA production from glucose in humans has been reported to be approximately one-fortieth of that in rats because of low activity of ATP-citrate lyase in humans (Hoffmann et al., 1980), and thus it is further unlikely that obese people would experience a suppression of body fat accumulation by HCA except in the setting of an unphysiological high-carbohydrate and low-fat diet containing very high level of HCA. Actually, the results of weight loss by HCA intake in humans are very controversial (Heymsfield et al., 1998; Kovacs et al., 2001; Kriketos et al., 1999; Mattes and Bormann, 2000; Rothacker and Waitman, 1997; Sergio, 1988; Thom and Andrews, 1997; Westerterp-Plantenga and Kovacs, 2002).

HCA-containing *Garcinia* products have been on the market for more than 8 years with no adverse side effects reported so far (Ohia et al., 2002). The LD_{50} of HCA (Super CitriMax™, a calcium/potassium salt of 60% HCA) was greater than 5000 mg/kg BW when administered once orally via gastric intubation to fasted male and female albino rats (Ohia et al., 2002). There was also no evidence of acute systemic toxicity among rabbits that were dermally administered HCA at 2000 mg/kg BW (Ohia et al., 2002). The LD_{50} obtained after intraperitoneal and oral administration of HCA ((−)-hydroxycitrate trisodium salt) to mice was more than 2000 mg/kg BW and 4000 mg/kg BW, respectively (Sullivan and Triscari, 1977). Therefore, HCA-containing *Garcinia* products have been deemed to be safe.

However, as clearly demonstrated in this study, marked testicular atrophy was observed at autopsy in the groups given HCA at 154 and 102 mmol/kg diet, but not in the group given HCA at 51 mmol/kg diet. Similar testicular toxicity was also observed in Fischer 344 rats fed a diet containing high levels of HCA-containing *Garcinia cambogia* (personal communication; Sekita, S. 2004, and our unpublished data), and so the toxicity is not unique to the Zucker obese rats used in the present study. On histopathological examination, severe testicular injury was found in the 154 mmol HCA/kg diet group, characterized by marked degeneration (disappearance) and atrophy of germ cells. Similar findings were also observed in the 102 mmol HCA/kg diet group, but with less severity.

This is the first published report of the testicular toxicity of HCA-containing *Garcinia cambogia*, and therefore, we do not currently know which of the constituents of this preparation is responsible for the toxicity. The *Garcinia cambogia* powder S® used in this study has been used as an ingredient of commercially available dietary supplements for so-called dieting, and accordingly, contamination with heavy metals and environmental pollutants can be ruled out. If we tentatively suppose that the toxicity is caused by HCA, its toxic or non-toxic cutoff point exists between 102 and 51 mmol HCA/kg diet, and the lower level can be seemed to be the NOAEL. The average intake of HCA in the 102 mmol/kg diet group through the entire experiment was 778 mg HCA/kg BW/d (Table 2), and 778 mg are nearly the same amount as the lower limit of the recommended intake range of 750–1500 mg HCA/d per person in commercially available dietary supplements containing *Garcinia cambogia* in Japan (Hayamizu et al., 2001; Sawada et al., 1997). The NOAEL of 51 mmol HCA/kg diet corresponds to 389 mg HCA/kg BW/d (Table 2), and the recommended intake range for a subject with body weight 50 kg is 15–30 mg HCA/kg BW/d, which is one-twentysixth to one-thirteenth of the NOAEL. Therefore, safety for human consumption is not essentially ruled out. However, a variety of *Garcinia*

cambogia-containing so-called health foods are sold on the market, and the tablet- and capsule-type dietary supplements are easily taken in excess. Therefore, we cannot recommend their use based at least on ineffectiveness particularly in people with higher lipogenic properties.

Weight reduction of the testis in experimental animals, including testicular atrophy and degeneration in some cases, has been observed in high/excess intake of methylxanthines occurring in cocoa powder (Tarka et al., 1991), alcohol (Klassen and Persaud, 1978; Shirai and Ikemoto, 1992) and tea catechins (Satoh et al., 2002). This phenomenon has also been noticed in dietary imbalance and nutritional restriction, such as essential fatty acid deficiency (Leat et al., 1983), a protein-free, carbohydrate-free or low-fat diet (Brinkworth et al., 1992), and severe feed restriction (Levin et al., 1993). Hence, testis weight reduction may not be rare phenomenon in high/excess and/or unbalanced intake of food components. Currently, we are energetically conducting studies to elucidate the mechanisms underlying this toxicity. At least as shown in Table 6, the concentrations of Leydig cell-releasing testosterone and pituitary LH did not change, Sertoli cell-releasing inhibin-B concentrations decreased, and pituitary FSH concentrations increased in the highest and second highest HCA groups. Although abnormal findings were not seen morphologically even in Sertoli cells as well as Leydig cells (Table 4 and Fig. 1), subnormal Sertoli cell function and/or spermatogenesis is reported to result in elevated FSH level and lowered inhibin-B level (Pierik et al., 2003). Therefore, the plasma hormonal changes (Table 6) and histopathological findings (Table 4 and Fig. 1) support subnormal Sertoli cell function and/or derangement of spermatogenesis. Further detailed mechanisms of HCA-containing *Garcinia cambogia*-induced testicular toxicity remain to be solved.

In conclusion, the high dose of HCA-containing *Garcinia cambogia* (154 mmol HCA/kg diet) was effective in suppressing epididymal fat accumulation even in developing male Zucker obese rats. However, marked testicular atrophy and toxicity were observed at 778 mg HCA/kg BW/d (102 mmol HCA/kg diet) and higher, but not at 389 mg HCA/kg BW/d (51 mmol HCA/kg diet), and thus this level was deemed to be the NOAEL.

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