Postprandial Thermic Effect of Chicken Involves Thyroid Hormones and Hepatic Energy Metabolism in Rats

Jun-ichi Wakamatsu1, Naomasa Takabayashi1, Misako Ezoe1, Takanori Hasegawa2, Tatsuya Fujimura2, Yoshihisa Takahata1, Fumiki Morimatsu2 and Takanori Nishihara1

1 Hokkaido University, Kita 9 Nishi 9, Kita-ku, Sapporo, Hokkaido 060–8589, Japan
2 Nippon Meat Packer, Inc., R & D Center, 3–3, Midorigahara, Tsukuba, Ibaraki 300–2646, Japan

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Summary We investigated the postprandial thermic effect of chicken and its mechanisms in rats. A chicken diet showed a strong thermic effect after consumption, and the removal of fat induced more rapid and stronger thermogenesis. Although thermogenesis induced by a purified chicken protein diet was also strong, the thermic reaction was not so rapid and a remarkable rise of peripheral temperatures was not observed. Defatted chicken and purified chicken protein activated the thyroid hormone system and up-regulated rate-limiting enzyme genes of glucose metabolism and the tricarboxylic acid (TCA) cycle in the liver. Moreover, chicken protein up-regulated the mRNA expression of a rate-limiting enzyme of hepatic lipid metabolism. It is possible that the mechanisms by which body temperature is raised are different between chicken protein and defatted chicken. On the other hand, it is possible that chicken fat suppressed the expression of energy metabolism-related genes that was induced by the consumption of lean chicken. As a result, a rise of postprandial body temperature might not have been induced after consumption of chicken fat. These results suggest that the consumption of lean chicken activates the thyroid hormone system and hepatic energy metabolism and consequently induces the postprandial thermic effect of chicken.

Key Words chicken, thermogenesis, thyroid hormone, energy metabolism

“Thermic effect of food” is defined as the increase in metabolic rate after ingestion of a meal (1) and also refers to diet-induced thermogenesis or a specific dynamic action. It has been shown that fat and carbohydrates have little thermic effect but that protein has a strong thermic effect (2–4). Moreover, strong thermic effects of capsaicin (5–7), caffeine (7, 8), ephedrine (7), ginger (9), black pepper (8), garlic (10) and green tea (7, 8, 11) have been reported.

Chinese nutritional therapy focuses on the qualitative effects of foods on the body, and foods are assigned on the basis of energetic classification primarily to four areas: thermal nature, flavor, organ network, and direction of movement (12, 13). As for the thermal nature, energetically “hot” or “warm” foods have a heating or warming effect on the body, whereas “cold” or “cool” foods cool the body. “Neutral” foods do not change the energy level of the body. The heating effect of “hot” or “warm” foods seems to resemble the thermic effect of food, but it is still unclear. In our previous study, it was found that mutton and venison, which are classified as “warm” foods, facilitated postprandial thermogenesis in rats in contrast to pork, beef (“neutral”) and rabbit meat (“cold”) (14). Examination of mutton constituents revealed that lean mutton protein contributed to the rise in the body temperature of rats. It was also found that lean mutton protein facilitated the secretion of thyroid hormones and the up-regulation of mRNA expression of several signaling molecules that are involved in energy metabolism in brown adipose tissue. These findings suggested that thermic effects were different among meat species according to the classification of their thermal nature and that the qualitative differences among meats affected various reactions in the body. However, it is still unclear why thermic effects are different among meat species. Chicken, which is widely consumed, is classified as a “warm” food in thermal nature. Consumption of chicken essence extract has been reported to increase metabolic rate (15, 16), but the mechanism of this thermic effect and the thermic effects of other chicken constituents have not been investigated.

Diet-induced thermogenesis is thought to be very similar to non-shivering thermogenesis (NST) (17). NST usually occurs in brown adipose tissue, which is abundant in small rodents and in infants, but contributes little to thermogenesis in adult humans. The liver, which plays a major role in metabolism, is a main heat-generating organ under a resting condition (18) and is also an important source of NST (19, 20). Indeed, it has been reported that the liver may have a significant role in diet-induced thermogenesis (21). However, it has not been determined whether the liver is involved in the postprandial thermic effect of meat.

Metabolism and thermogenesis are controlled by cat-
echolamines, adrenocorticotropic hormone and thyroid hormones, which are secreted by the directive of the hypothalamus, and the main regulatory factor of NST is noradrenaline (norepinephrine). Although mutton, especially lean mutton protein, seemed to have the strongest thermic effect among meat species examined in our previous study, levels of catecholamines including noradrenaline were not significantly different (14). We also found that consumption of lean mutton protein stimulates the secretion of thyroid hormones and facilitates energy metabolism in rats. Thyroid hormones have long been accepted to be an important determinant of overall energy expenditure and basal metabolic rate (22, 23). Effects of thyroid hormones on diet-induced thermogenesis have also been studied (24–26). Recently, it has been reported that a soy protein diet alters not only thyroid hormone metabolism but also the expression of hepatic genes regulating fatty acid in rats (27).

MATERIALS AND METHODS

Animals and diets. The experimental plan of this study was approved by the Laboratory Animal Care Committee of Hokkaido University. The animal experiment was carried out according to our previous study (14). Five-week-old male Wistar rats were commercially obtained from Japan Laboratory Animals Inc. (Tokyo, Japan). The animals were housed individually in stainless steel cages with a 12-h light-dark cycle (lights on from 10:00 to 22:00) in an isolated room at a controlled temperature (22–24˚C) and humidity (40–60%). Before starting the experiment, all animals were acclimated to the AIN-93G diet except for replacement of soybean oil with corn oil for 1 wk. Animals had free access to feed and water. After fasting for 18 h, the experimental diets (7 g/animal) were given to animals in the last 2 h of a dark cycle (from 8:00 to 10:00).

Chicken and mutton legs were purchased from a domestic market. The fractionation of meat was performed as described previously (14). First, as much fat and connective tissue as possible were removed from the meat and then minced meat was freeze-dried (freeze-dried chicken (FDC) and freeze-dried mutton (FDM)). Next, defatted chicken (DFC) was prepared from FDC with n-hexane, and chicken fat (CF) and mutton tallow were extracted from adipose tissue of chicken and mutton with n-hexane. Moreover, purified chicken protein (PCP) was washed from DFC with hot ethanol and boiled water according to our previous report (14). Protein contents of FDC, DFC, PCP and FDM were 77.5, 86.9, 98.3 and 75.5%, respectively. Fat contents of FDC and FDM were not detected (<0.1%). Based on AIN-
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...9 3G except for the replacement of soybean oil with corn oil, experimental diets (whole chicken (WC), CF, DFC, PCP, whole mutton (WM) and control (Ctrl)) were prepared (Table 1). Casein and corn oil were used in the Ctrl group. Contents of protein and fat were the same in the groups.

**Measurements of body weight and body temperature.** General preparation was performed as described previously (14). The rats were anesthetized with an intraperitoneal injection of urethane (700 mg/kg) and α-chloralose (60 mg/kg) immediately after 2 h of feeding. The body weight and body temperature of each animal were measured under anesthesia. To exclude the effect of heat dissipation, body temperatures were measured on a thermostatic plate (33˚C, KN-210-6, Natsume Seisakusho Co. Ltd., Tokyo, Japan) in a room with controlled temperature (33˚C every 30 min after feeding for 2 h. Rectal temperature was measured using a thermometer (CT-1307, Custom, Tokyo, Japan) with a thermocouple (1600K-T21-ASP, Anritsu Meter Co. Ltd., Tokyo, Japan). The thermocouple was gently inserted 2 cm into the rectum after dipping it in glycerol. Back temperature was measured in the interscapular area using an infrared thermometer (IT-550, Horiba, Ltd., Kyoto, Japan) after the back hair had been clipped short with hair clippers. Measurements of concha and sole temperatures were also performed by using an infrared thermometer (IT-550, Horiba, Ltd.,). Thermogram images of each animal were obtained by using a thermograph camera (Handy Thermo TVS-200, Nippon Avionics Co., Ltd., Tokyo, Japan) immediately after the measurement of body temperature. Urethane-chloralose anesthesia was added if needed.

**Blood and tissue collection.** Blood was collected from the inferior vena cava under anesthesia and plasma was immediately prepared in plastic tubes containing EDTA. The collected blood was kept at room temperature for 30 min for coagulation and then kept on ice for 30 min. Serum was obtained from the coagulated blood by centrifugation at 1,940 g for 30 min at 4˚C. The liver serum and plasma measurements. Serum glucose (Clinitest kit, Wako Pure Chemical Industries, Ltd., Tokyo, Japan), triglyceride (TG) (E-test kit, Wako Pure Chemical Industries Ltd.) and free fatty acid (FFA) (C-test kit, Wako Pure Chemical Industries Ltd.) and glucagon (Glucagon (Rat) EIA Kit, Phoenix Pharmaceuticals, Inc., Burlingame, CA) and insulin (Rat Insulin ELISA kit (AKRIN-010T), Shibayagi Co. Ltd., Gunma, Japan) were measured by using commercial assay kits. Analysis of adrenaline, noradrenaline and dopamine in plasma were entrusted to a clinical laboratory organization, BMI Inc. (Tokyo, Japan), which used a standardized analytical method (HPLC method). Total triiodothyronin (T3) and total thyroxin (T4) in plasma were measured by the CLIA method (BML Inc.).

**Analysis of mRNA.** Total RNA of the liver was isolated with ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer’s protocol. Each mRNA expression was analyzed by quantitative RT-PCR using a LightCycler instrument (Roche Diagnostics, Basel, Switzerland). Fast-strand cDNA was synthesized in 20 µL reverse transcriptase (RT) reaction buffer from 1 µg of total RNA using a Transcriptor First-Strand cDNA Synthesis Kit (Roche Diagnostics) according to the manufacturer’s protocol. PCR was performed with 1 µL of RT reaction buffer using a LightCycler FirstStart DNA Master SYBR Green Kit (Roche Diagnostics) or LightCycler FirstStart DNA MasterPLUS SYBR Green Kit (Roche Diagnostics). The primers for glucokinase (GK) were 5’-ATGAAGACCGCACAATGAGC-3’ (forward) and 5’-CATCCACCATCGGGTACTAC-3’ (reverse). The real-time PCR conditions for amplification were denaturation at 95˚C for 10 s, annealing at 57˚C for 5 s, and extension at 72˚C for 45 cycles. The primers for phosphorfructokinase (PFK) were 5’-TTACCGAGATTCACTGTTCC-3’ (forward) and 5’-CCACAGTTGCTCTGTCGTA-3’ (reverse). The real-time PCR conditions for amplification were denaturation at 95˚C for 10 s, annealing at 58˚C for 5 s, and extension at 72˚C for 9 s for 45 cycles. The primers for pyruvate kinase (PK) were 5’-AGAGATCTTCCCCGTGCT-3’ (forward) and 5’-ACCCTGACCAACACTACCA-3’ (reverse). The real-time PCR conditions for amplification were denaturation at 95˚C for 10 s, annealing at 60˚C for 5 s, and extension at 72˚C for 15 s for 45 cycles. The primers for carnitine palmitoyltransferase 1a (CPT1a) were 5’-GGAGACAGA-CACCATCACAATATA-3’ (forward) and 5’-AGGTGATG-GACTTGCTAACC-3’ (reverse). The real-time PCR conditions for amplification were denaturation at 95˚C for 10 s, annealing at 55˚C for 5 s, and extension at 72˚C for 16 s for 45 cycles. The primers for citrate synthase (CS) were 5’-CCCGTGCTATGACCTGGGCTCT-3’ (forward) and 5’-CCCTGGCCCAACGAGATGTC-3’ (reverse). The real-time PCR conditions for amplification were denaturation at 95˚C for 10 s, annealing at 60˚C for 5 s, and extension at 72˚C for 8 s for 45 cycles. The primers for thyroid hormone responsive Spot 14 protein (THRSP) were 5’-AGCTGTCTCCCTCCATCT-3’ (forward) and 5’-GGTTCTAGGTTCCAGCTCT-3’ (reverse). The real-time PCR conditions for amplification were denaturation at 95˚C for 10 s, annealing at 56˚C for 5 s, and extension at 72˚C for 10 s for 45 cycles. The primers for glucose-6-phosphate dehydrogenase (G6PD) were 5’-AGCTGTCTATCCTGGTGACG-3’ (forward) and 5’-TGACATTGTCAAGTCCAGCTC-3’ (reverse). The real-time PCR conditions for amplification were denaturation at 95˚C for 10 s, annealing at 54˚C for 5 s, and extension at 72˚C for 10 s for 45 cycles. The primers for aldolase (ALD) were 5’-GAGGCAGCAATGAGATG-3’ (forward) and 5’-CGTTCAGGTCCAGCTCTC-3’ (reverse). The real-time PCR conditions for amplification were denaturation at 95˚C for 10 s, annealing at 52˚C for 5 s, and extension at 72˚C for 15 s for 45 cycles. The primers for lactate dehydrogenase (LDH) were 5’-AGCTGTCTATCCGTGGACG-3’ (forward) and 5’-TGACATTGTCCAGCTC-3’ (reverse). The real-time PCR conditions for amplification were denaturation at 95˚C for 10 s, annealing at 52˚C for 5 s, and extension at 72˚C for 15 s for 45 cycles.

**Statistical analysis.** Values are given as means±SE. All data were analyzed by parametric one-way analysis.
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of variance (ANOVA) and then by Tukey’s multiple comparison test when appropriate (Excel toukei 2006, Social Survey Research Information Co., Ltd., Tokyo, Japan). p values <0.05 were considered as statistically significant.

RESULTS

Postprandial change in body temperature of rats fed the chicken diet

Postprandial body temperatures rose up to 2 h after feeding time (2 h), i.e., up to 4 h after the beginning of feeding, in all groups and in all parts (Fig. 1). Body temperature in the WC group remained as high as that in the WM group and was significantly higher than that in the fasting group. Mutton has been reported to have a strong thermic effect among animal meats (14), and it was shown that chicken also has a strong postprandial thermic effect. Body temperatures reached plateaus from 2 h after feeding time. Although no significant difference in food intake was observed between the WC group and the WM group, body weight in the fasting group was significantly lower than those in the WC and WM groups because of no diet (Table 2).

Table 2. Food intake during 2 h of feeding and body weights of rats fed the experimental diets.

<table>
<thead>
<tr>
<th></th>
<th>WC$^1$</th>
<th>WM$^1$</th>
<th>Fast</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>$5.8\pm0.5$</td>
<td>$5.1\pm1.0$</td>
<td>--</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>$187\pm2^a$</td>
<td>$182\pm3^a$</td>
<td>$171\pm2^b$</td>
</tr>
</tbody>
</table>

Values are means±SE. Means in a column without a common letter (a, b) differ, p<0.05.$^1$ WC: whole chicken, WM: whole mutton.

Postprandial change in body temperature of rats fed a diet containing fractionated chicken

Next, the postprandial change in body temperatures of rats that were fed diets containing fractionated chicken were examined under anesthesia every 30 min from 1 h after feeding time (Fig. 2A–D). All body temperatures in the DFC group after 1 h of feeding time were significantly higher than those in the other groups. Back and concha temperatures in the DFC group were

Fig. 1. Changes in postprandial body temperatures (A: rectal, B: back, C: concha, D: sole) of rats fed diets containing chicken (WC: whole chicken) or mutton (WM: whole mutton). After 2 h of feeding, each body temperature was measured under anesthesia. Values are means (n=6–7 rats per diet group). Means not sharing a common letter within the same time are not significantly different between groups (p<0.05).

Table 2. Food intake during 2 h of feeding and body weights of rats fed the experimental diets.

<table>
<thead>
<tr>
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<th>WC$^1$</th>
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<td>11</td>
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</tr>
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</tr>
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</tr>
</tbody>
</table>

Values are means±SE. Means in a column without a common letter (a, b) differ, p<0.05.$^1$ WC: whole chicken, WM: whole mutton.
remarkably higher than those in the other groups. Thus, the removal of fat from chickens induced more rapid and stronger thermogenesis. On the other hand, body temperatures in the CF group did not rise from 1.5 h after feeding time. Rectal temperatures in the Ctrl, WC and PCP groups rose linearly up to 2 h after feeding time. Back temperature in the WC group rose linearly up to 2 h after feeding time, but that in the PCP group rapidly rose from 1 h to 1.5 h after feeding time. Concha temperatures increased significantly up to 1.5 h after feeding time in all groups except for the DFC group. Sole temperatures in the Ctrl, WC and PCP groups rose linearly up to 2 h after feeding time. However, the increase in sole temperature in the Ctrl group was slightly less than that in the WC and PCP groups. Back and concha temperatures in the CF group after 2 h of feeding time

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**Fig. 2.** Changes in postprandial body temperatures (A: rectal, B: back, C: concha, D: sole) and thermograms (E) of rats fed diets containing fractionated chicken (WC: whole chicken, CF: chicken fat, DFC: defatted chicken, PCP: purified chicken protein). After 2 h of feeding, each body temperature and thermograms were obtained under anesthesia. The back hair of the rat was clipped with a hair clipper before imaging. Increases of temperature in the limbs (arrow) and trunk (asterisk) are shown. Values are means ± SE (n=7–9 rats per diet group). ab Means not sharing a common letter within the same group are not significantly different between groups (p<0.05). xyz Means not sharing a common letter within the same time are not significantly different between groups (p<0.05).
were significantly lower than those in the WC, DFC and PCP groups. No significant difference in food intake or body weight was observed among the groups (Table 3).

Thermograms of typical rats in the groups after 2 h of feeding time are shown in Fig. 2E. A rise of body temperature, especially in the trunk, was observed in the WC, CF and DFC groups (Fig. 2E, arrows) and these results coincided with results for sole temperatures (Fig. 1). Moreover, rises in peripheral temperatures such as temperatures in limbs were observed in the WC and DFC groups (Fig. 2E, asterisks). These data suggested that thermogenesis induced by the purified chicken protein system. On the other hand, the T3 level in the PCP group was significantly higher than the levels in the WC, CF and DFC groups, but the T4 level in the PCP group was also high (Fig. 3C). However, the expression level of PFK mRNA in the DFC group was significantly lower than that in the DFC group. Thus, it was thought that chicken-induced thermogenesis was not caused by stimulation of the sympathetic nervous system. On the other hand, the T1 level in the PCP group was significantly higher than the levels in the WC, CF and DFC groups, but the T1 level in the PCP group was significantly lower than that in the DFC group.

Expression of metabolic enzyme genes in the liver

The mRNA expression levels of several rate-limiting enzymes in the hepatic metabolic pathway were measured. First, mRNA expressions levels of GK, PFK and PK, which are rate-limiting enzymes in glycolysis, were measured after 2 h of feeding time. The expression levels of GK mRNA in the DFC group tended to be higher than the levels in other groups, but there was no significant difference among the groups (Fig. 3A). On the other hand, the expression level of PFK mRNA in the PCP group was significantly higher than that in the CF group, and the level in the DFC group was also high (Fig. 3B). The expression level of PK mRNA in the DFC group was significantly higher than that in the Ctrl group, and the level in the PCP group was also high (Fig. 3C). How-

Table 3. Food intake during 2 h of feeding and body weights of rats fed the experimental diets.

<table>
<thead>
<tr>
<th></th>
<th>Ctrl</th>
<th>WC</th>
<th>CF</th>
<th>DFC</th>
<th>PCP</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>6.4±0.2</td>
<td>5.9±0.3</td>
<td>6.1±0.3</td>
<td>6.1±0.3</td>
<td>6.1±0.2</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>169±5</td>
<td>175±5</td>
<td>170±3</td>
<td>178±4</td>
<td>164±2</td>
</tr>
</tbody>
</table>

Values are means±SE.

1 WC: whole chicken, CF: chicken fat, DFC: defatted chicken, PCP: purified chicken protein.

Table 4. Blood biochemical parameters in rats fed the experimental diet including fractionized chicken after 2 h of feeding.

<table>
<thead>
<tr>
<th></th>
<th>Ctrl</th>
<th>WC</th>
<th>CF</th>
<th>DFC</th>
<th>PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>167.3±3.6ab</td>
<td>162.2±6.5ab</td>
<td>177.7±5.1a</td>
<td>155.5±7.0ab</td>
<td>152.7±4.0b</td>
</tr>
<tr>
<td>TG, mg/dL</td>
<td>88.8±9.0</td>
<td>73.0±7.4</td>
<td>78.9±6.5</td>
<td>77.7±11.0</td>
<td>69.3±3.2</td>
</tr>
<tr>
<td>FFA, mEq/dL</td>
<td>0.73±0.07</td>
<td>0.76±0.06</td>
<td>0.77±0.06</td>
<td>0.83±0.06</td>
<td>0.81±0.08</td>
</tr>
<tr>
<td>3-HHA, μmol/mL</td>
<td>32.6±1.4ab</td>
<td>36.6±8.7a</td>
<td>29.9±3.5a</td>
<td>32.4±8.9a</td>
<td>70.4±23.3a</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>1.76±0.21</td>
<td>2.99±0.42</td>
<td>2.61±0.48</td>
<td>2.38±0.34</td>
<td>1.70±0.18</td>
</tr>
<tr>
<td>Glucagon, ng/mL</td>
<td>0.97±0.05b</td>
<td>1.36±0.13ab</td>
<td>1.22±0.07ab</td>
<td>1.32±0.15ab</td>
<td>1.79±0.22a</td>
</tr>
<tr>
<td>Adrenaline, ng/mL</td>
<td>0.98±0.26</td>
<td>0.75±0.19</td>
<td>0.71±0.27</td>
<td>0.35±0.07</td>
<td>0.58±0.16</td>
</tr>
<tr>
<td>Noradrenaline, ng/mL</td>
<td>0.69±0.07</td>
<td>0.63±0.11</td>
<td>0.57±0.10</td>
<td>0.56±0.14</td>
<td>0.86±0.18</td>
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<tr>
<td>Dopamine, ng/mL</td>
<td>0.05±0.01ab</td>
<td>0.06±0.01ab</td>
<td>0.06±0.01ab</td>
<td>0.04±0.01a</td>
<td>0.10±0.01b</td>
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<tr>
<td>T3, ng/dL</td>
<td>64±2bc</td>
<td>56±2ab</td>
<td>54±1a</td>
<td>56±2ab</td>
<td>71±2c</td>
</tr>
<tr>
<td>T4, μg/dL</td>
<td>3.6±0.1ab</td>
<td>3.7±0.1ab</td>
<td>3.4±0.1ab</td>
<td>3.8±0.1a</td>
<td>3.2±0.1b</td>
</tr>
</tbody>
</table>

Values are means±SE, n=7–9. Means in a row without a common letter (a, b, c) differ, p<0.05.

1 WC: whole chicken, CF: chicken fat, DFC: defatted chicken, PCP: purified chicken protein.
ever, the expression levels of these glycolytic rate-limiting enzymes in the WC group, in which body temperature was higher than those in the CF and Ctrl groups, did not increase compared with those in the DFC and PCP groups.

The expression level of CPT1a mRNA in the liver of rats in the PCP group was the highest among groups and was significantly higher than in the DFC and Ctrl groups and tended to be higher than that in the WC group (Fig. 3D).

Expression of thyroid hormone target genes in the liver
Since the liver is a major organ of thermogenesis at rest (28, 29), the expression levels of thyroid hormone target genes in the liver were measured after 2 h of feeding time. The CS mRNA expression levels in the DFC and PCP groups were significantly higher than those in the Ctrl and CF groups and tended to be higher than that in the WC group (Fig. 3E).

![Fig. 3. Effects of fractionated chicken diets (WC: whole chicken, CF: chicken fat, DFC: defatted chicken, PCP: purified chicken protein) on mRNA expression of energy metabolism-related rate-limiting enzymes (A: glucokinase (GK), B: phosphofructokinase (PFK), C: pyruvate kinase (PK), D: carnitine palmitoyltransferase 1a (CPT1a), E: citrate synthase (CS)) in the rat liver. After 2 h of feeding and 2 h of rest, livers were collected. Liver transcripts were normalized to β-actin mRNA. Values are means±SE (n=7–9 rats per diet group). Means not sharing a common letter within the same group are not significantly different between groups (p<0.05).](image)

![Fig. 4. Effects of fractionated chicken diets (WC: whole chicken, CF: chicken fat, DFC: defatted chicken, PCP: purified chicken protein) on the expression of thyroid hormone target genes (A: thyroid hormone responsive Spot 14 protein (THRSP), B: glucose-6-phosphate dehydrogenase (G6PD)) in the rat liver. After 2 h of feeding and 2 h of rest, livers were collected. Liver transcripts were normalized to β-actin mRNA. Values are means±SE (n=7–9 rats per diet group). Means not sharing a common letter within the same group are not significantly different between groups (p<0.05).](image)
DFC group was significantly higher than those in the C and PCP groups (Fig. 4A). The expression pattern of the G6PD gene among groups was similar to that of the THRSP gene, and there was no significant difference (Fig. 4B).

**DISCUSSION**

The body temperature of rats that were fed the chicken diet was as high as that of rats that were fed the mutton diet. The postprandial thermic effect of chicken persisted for a few hours as did that of mutton. Mutton has been reported to have a strong postprandial thermic effect among animal meats (14). Therefore, the postprandial thermic effect of chicken also appears to be strong. Regarding fractionated chicken, the body temperature in the CF group was lower than those in the WC, DFC and PCP groups, in which lean chicken was consumed. The body temperature in the DFC group rose rapidly after consumption compared with that in the other groups. However, the body temperature in the DFC group was as high as those in the WC and PCP groups at 2 h after feeding time. These results suggest that not chicken fat but lean chicken has a strong postprandial thermic effect. The mechanisms by which body temperatures are raised seemed to be different for the purified chicken protein and the extract from lean chicken.

Even though the purified chicken protein was highly purified, the back temperature at 2 h after feeding time was higher than that in the CF group. Serum glucose level in the PCP group was lower than the levels in the low body temperature groups (Ctrl and CF groups), and the mRNA expression levels of glycolytic rate-limiting enzymes such as PFK and PK in the PCP groups were higher than the levels in the low body temperature groups. There was no significant difference in plasma insulin level among groups but the plasma glucagon level in the PCP group was higher than those in the low body temperature groups. It is possible that secretion of glucagon was facilitated since serum glucose was decreased due to glycolysis. The 3-HBA level in the PCP group was remarkably higher than the levels in the other groups. There was no significant difference in TG levels, but the TG level in the PCP group was the lowest among the groups. Moreover, the mRNA expression level of CPT1α, a rate-limiting enzyme in lipid metabolism, was remarkably higher in the PCP group than in the other groups. In addition, the mRNA expression level of CS, which is a rate-limiting enzyme of the tricarboxylic acid cycle (TCA cycle), in the PCP group was significantly higher than the levels in the Ctrl and CF groups. On the other hand, the plasma T3 level in the PCP group was not so high, but the plasma T3 level was the highest among all groups. Thyroid hormones act to increase the basal metabolic rate and lead to heat production. However, the mRNA expression levels of thyroid hormone target genes in the PCP group were not higher than those in the DFC group. Several researchers have reported the effects of dietary protein on thyroid hormone action. Soy protein feeding raised the plasma T3 level (30, 31). Red meat (kangaroo) and whey protein did not affect the plasma T4 level (32). In our previous study, consumption of mutton meat protein was shown to accelerate the secretion of thyroid hormones, resulting in a rise of body temperature (14). Therefore, our results suggest that thyroid hormone secretion due to chicken protein consumption up-regulates glycolysis, lipid metabolism and the TCA cycle in the liver and consequently raises body temperature.

Since defatted chicken consumption induced both rapid and strong thermogenesis and rise in peripheral body temperature, the ethanol- and water-extract from lean chicken seems to be involved in the reactions. Plasma thyroid hormone levels in the DFC group did not show remarkable changes, but the mRNA expression level of THRSP in the DFC group was significantly higher than those in the WC and PCP groups. The THRSP gene is well known as a hepatic product induced rapidly by thyroid hormones (33, 34), and the greatest increase in expression levels after thyroid hormone treatment was shown by hepatic cDNA microarray analysis (35). THRSP is thought to be a modulator of fatty acid synthesis (36). The mRNA expression pattern of G6PD was also similar to that of THRSP. G6PD is the first and rate-limiting enzyme in the pentose phosphate pathway (37), which plays multifunctional roles to generate reduced nicotinamide adenine dinucleotide phosphate (NADPH) and ribose-5-phosphate for biosynthesis of nucleotide and nucleic acid and to supply energy. These results suggest that thyroid hormone signaling is involved in the thermic effect of defatted chicken. On the other hand, the mRNA expression levels of glycolytic rate-limiting enzymes including GK and PK in the DFC group were higher than those in the Ctrl, WC and CF groups. Serum glucose levels in the DFC group were lower than those in the low body temperature groups (Ctrl and CF groups). CS mRNA expression level in the DFC group was significantly higher than the levels in the Ctrl and CF groups, but the mRNA expression level of CPT1α was as low as the levels in the CF, WC and Ctrl groups. It has been reported that the consumption of chicken essence increased metabolic rate (15) and resting energy expenditure (REE) values (16). Since those essences are almost the same as the ethanol- and water-extracts in this study, the results of those studies support our results. Multifunctional bioactivities of chicken essence have recently been reviewed (38). Carnosine, anserine and taurine, which are abundantly included in chicken, have been reported to be involved in blood flow and blood pressure changes (39–42). Our findings suggest that defatted chicken facilitates both thyroid hormone signaling and energy metabolism except for lipid metabolism. However, the metabolic rate-limiting enzymes that were up-regulated by the ethanol- and water-extract from lean chicken were different from those that were up-regulated by the chicken protein. Further studies are needed to elucidate the reason for this.

Body temperatures in the CF group were the lowest among the groups fed fractionated chicken. The body temperature in the WC group at 1 h after feeding time
was significantly lower than that in the DFC group, in which chicken fat was not consumed. Chicken fat may therefore suppress the postprandial thermogenesis of chicken, especially the ethanol- and water-extract from lean chicken, resulting in suppression of the rise in body temperature. The mRNA expression levels of most energy metabolic rate-limiting enzymes (PFK, PK, CPT1a and CS) in the WC group were lower than those in the DFC and PCP groups. Moreover, plasma thyroid hormone levels in the WC and CF groups were relatively low among the groups, and the mRNA expression level of THRSP in the WC group was lower than that in the DFC group. Fatty acids have various functionalities, and polyunsaturated fatty acids have been reported to improve blood flow, glycolysis and lipid metabolism. On the other hand, saturated fatty acids have been reported to suppress blood flow and lipid metabolism (43, 44). Indeed, chicken fat is classified as “cold” food in thermic nature and it is thought that the body is cooled after consumption (13). The Japanese persimmon (kaki), which is also classified as “cold” food in thermal nature, has been shown to keep body temperature lower at the feet and wrists and to decrease blood flow at the wrists (45). Thus, it is possible that these foods have specific functions to lower postprandial body temperature. Our results suggest that chicken fat consumption suppresses the signaling of thyroid hormones and consequently suppresses the energy metabolism. However, it is still unclear how chicken fat consumption suppresses the thermic effect of lean chicken, including chicken protein and ethanol- and water-extract.

In summary, we have shown that lean chicken mainly induced the postprandial thermic effect of chicken. The results suggested that lean chicken facilitated the secretion of thyroid hormones and up-regulated the mRNA expression of hepatic rate-limiting enzymes in energy metabolism, especially glycolysis and the TCA cycle. Consequently, chicken protein and ethanol- and water-extract from lean chicken mainly raised the temperatures of the trunk and limbs, respectively. Since various constituents in lean chicken may intricately contribute to various reactions in the thermic effect of chicken, we could not narrow down the candidates. On the other hand, chicken fat is thought to inhibit the above-mentioned thermic effect of lean chicken to some extent. In any case, the postprandial thermic effect of chicken is as high as that of mutton. We concluded that thyroid hormones contribute to the postprandial thermic effect of chicken similarly to mutton. However, since blood analysis and mRNA expression analyses were performed only at 2 h after feeding time in this study, time-course studies and protein expression analyses are needed to elucidate the detailed mechanism.

REFERENCES

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