Radiotelemetric characterization of overweight-associated rises in blood pressure and heart rate

HEINZ RUPP AND BERNHARD MAISCH
Molecular Cardiology Laboratory, Department of Internal Medicine and Cardiology, Philipps University of Marburg, 35033 Marburg, Germany

Rupp, Heinz, and Bernhard Maisch. Radiotelemetric characterization of overweight-associated rises in blood pressure and heart rate. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1540–H1545, 1999.—We addressed the hypothesis that hypercaloric diets induce hyperkinetic hypertension irrespective of day-night cycle and locomotor activity that is associated with altered cardiac myosin isozymes. Normotensive rats with implanted radiotelemetry pressure transducers were fed increasing amounts of coconut fat (8, 16, and 24% each for 2 wk) corresponding to 20–47% of total calories from fat. Thereafter, increasing amounts of sucrose (16, 32, and 50%) and fructose (50%) were added to the 24% fat diet corresponding to 13–40% of total calories from sugar. In contrast to the fat diets, the 32% and 50% sucrose diets as well as the 50% fructose diets increased (P < 0.05) blood pressure (systolic maximum +13 mmHg, diastolic maximum +4 mmHg, mean maximum +7 mmHg) and heart rate (maximum +50 beats/min) irrespective of the day-night cycle and the unaltered locomotor activity. Furthermore, body weight increased (P < 0.05) during the 32% and 50% sucrose feedings. The increased blood pressure and heart rate normalized after rats were fed a regular chow. We concluded that an excessive caloric intake results in hyperkinetic hypertension that increases the myosin V1; proportion.

hy- pertension; sucrose; fructose; fat; myosin

LIFESTYLE FACTORS such as dietary intake have an important influence on hypertension, coronary artery disease, stroke, and diabetes mellitus (9). Of relevance are dietary practices leading to weight gain caused by an energy intake that exceeds energy expenditure (5, 12, 15). Emphasis has been placed on the direct influences of an excess intake of fat and simple sugars. Variations in the prevalence of coronary artery disease thus correlated with the proportion of calories derived from saturated fat (9), suggesting dietary influences beyond those of cholesterol (11, 13). In contrast to saturated fat, sugar intake did not qualify as a risk factor for coronary artery disease (4). The influence of a high sugar intake together with a high fat consumption remains, however, unclear. In addition to specific influences of macronutrients, the caloric intake per se could be important. Thus overweight-related hypertension is linked to metabolic disorders involving insulin resistance and sympathoadrenal activity (15). Although a link of hypertension with the overweight state has been documented (5, 15), initial processes during the manifestation of hypertension remain poorly defined (6, 7).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
2% C\textsubscript{18:0} (stearic acid), and 2% C\textsubscript{18:1}. Thereafter, rats were fed a 24% coconut fat diet that contained increasing amounts of sucrose (Südzucker, Oberursel, Germany), again on a 2-wk schedule. The sucrose content was 16% (13% of total calories) for weeks 7 and 8, 32% (26% of total calories) for weeks 9 and 10, and 50% (40% of total calories) for weeks 11–13. Finally, a 50% fructose (Seeberger, Ulm, Germany) and 24% coconut fat diet was fed (weeks 14 and 15). The diets were prepared every day using a 66% (wt/vol) solution of sucrose or fructose (dissolved at 40°C). Food intake was estimated daily and converted into energy equivalents using the following caloric contents: 9.3 kcal/g coconut fat, 4.1 kcal/g sucrose or fructose, and 3.3 kcal/g chow.

In a separate experiment, 10 rats were fed a chow mixed with 12% lard. Another 10 rats were fed a 12% lard-66% fructose diet, and an additional 10 rats were fed a 12% lard-66% fructose diet and treated daily with 50 mg/kg BM-13907 (provided by Dr. P. Freund, Boehringer Mannheim). After 7 wk, rats were killed by decapitation for monitoring of serum parameters and myosin isozymes. Experimental procedures were performed at the Physiological Institute of the University of Tübingen (Tübingen, Germany) and were approved by the Animal Care and Use Committee.

Radiotelemetry monitoring. The radiotelemetry system (Data Sciences, St. Paul, MN; Ref. 1) consisted of five TA11PA-C40 pressure transmitters for monitoring blood pressure (systolic, diastolic, and mean), heart rate, and locomotor activity and five RA 1010 receivers. Heart rate was derived from pressure recordings, and locomotor activity was assessed from the movement of a transmitter-implanted rat relative to the fixed position of the receiver aerial positioned under the rat’s cage.

Surgery was performed on rats under Nembutal (60 mg/kg ip) anesthesia. The catheter connected to the pressure transducer was implanted into the abdominal aorta 1–2 mm anterior to the bifurcation. The transmitter body was sutured around the catheter. The radiotelemetry system (Data Sciences, St. Paul, MN) was implanted into the abdominal aorta 1–2 mm anterior to the bifurcation. The transmitter body was sutured under the rat’s cage.

Data were collected for 30 s every 5 min, i.e., 288 30-s measurements/24 h for heart rate, blood pressure (systolic, diastolic, and mean), and locomotor activity. With the use of the DataQuest IV software (Data Sciences), values were averaged in the specified time intervals. Further analysis was performed using STATISTICA (StatSoft, Tulsa, OK). Data were divided into four time periods, two periods with the lights off (dark), when rats were predominantly asleep (1800–2400 and 2400–0600), and two periods with the lights on (light), when rats were predominantly awake (0600–1200 and 1200–1800). Data associated with a specified range of locomotor activity, i.e., zero or >80 (arbitrary units), were also analyzed (21). The activity polling rate was 64 Hz.

Serum parameters and myosin isozymes. After having been fed the 24% coconut fat-50% fructose diet for 2 wk, the five yoked rats (housed together with the pressure transducer-implanted rats) were killed by decapitation. The five rats with implanted pressure transducers were killed after having been fed a regular chow for 2 wk subsequent to the 24% coconut fat-50% fructose diet. Left ventricles were stored in liquid nitrogen. The first 2–3 ml of blood collected from the trunk were used for determination of serum parameters. Triglycerides were measured by the glycerolphosphate oxidase/peroxidase method (Boehringer Mannheim), insulin was measured by radioimmunoassay (Ciba-Corning, Giessen, Germany), and glucose was measured by the hexokinase/glucose-6-phosphate dehydrogenase method (Boehringer Mannheim). Triiodothyronine (T3) was determined by Dr. R. Wahl (Medical Clinic IV, University of Tübingen) using radioimmunoassays. Myosin isozyme populations were quantitated after separation by polyacrylamide gel electrophoresis in the presence of sodium pyrophosphate (18).

Statistical analysis. Statistical comparisons in the time-course studies were performed by ANOVA with repeated measures (randomized-block ANOVA) using STATISTICA. The effect of a given diet was assessed by comparing the values during the last 3 days preceding a given diet with the values 12, 13, and 14 days after the respective diet. Differences in body weight, serum parameters, and myosin isozymes were assessed by two-sided unpaired Student’s t-test. Multiple comparisons in the 12% lard-66% fructose-fed rats were made by ANOVA and Duncan’s new multiple range test. Data are presented as means ± SD. Statistical significance was assumed at P < 0.05.

RESULTS

Feeding experiments were started when rats exhibited a regular circadian rhythm 3 wk after the implantation of pressure transmitters. A characteristic feature was the sudden drop of locomotor activity during the light period (0600–1800). Comparable circadian influences were observed for heart rate as well as systolic and diastolic blood pressure. During feeding of the various diets, total caloric intake was assessed as well as calories derived from fat and sugar (Fig. 1A). Feeding of the 8, 16, and 24% fat diets, each for 2 wk, resulted in an increased total caloric intake, whereby statistical significance was not reached on each time point (Fig. 1A). Body weight was not significantly increased (Fig. 1B). The addition of 16% or 32% sucrose to the 24% coconut fat diet also increased caloric intake (Fig. 1A). Body weight was increased (P < 0.05) during feeding of the 32% and 50% sucrose diets (Fig. 1B). Exchanging 50% sucrose for 50% fructose resulted initially in a reduced caloric intake (Fig. 1A), which was associated with a reduction in body weight (Fig. 1B).

Dietary influences on blood pressure and heart rate as well as locomotor activity are shown in Fig. 2. To examine 24-h values, the daily 288 30-s measurements were averaged. Systolic blood pressure was not significantly altered after fat calories were increased from 20% (8% wt/wt fat) to 35% (16% wt/wt fat) and then 47% (24% wt/wt fat) (Fig. 2A). After the addition of sucrose to the diet corresponding to 13% sugar calories (16% wt/wt), systolic blood pressure was increased (P < 0.05) (Fig. 2A) and was raised further after the addition of sucrose corresponding to 26% (32% wt/wt sucrose) and then 40% (50% wt/wt sucrose) sugar calories (Fig. 2A).

Increasing the content of calories derived from fat from 20% to 35% and then 47% resulted in a transient rise in diastolic blood pressure that returned, however, to baseline values during the respective feeding interval (Fig. 2B). When sucrose was added corresponding to 26% and 40% sugar calories, diastolic blood pressure was consistently increased (P < 0.05) during the feed-
To examine whether fructose can have a selective influence on thyroid hormone and myosin isozymes, rats were fed a 12% lard-66% fructose (wt/wt) diet for 7 wk. In contrast to diets used for the radiotelemetric monitoring interval (Fig. 2B). Heart rate was also only increased during the feeding of 26% and 40% sugar (sucrose) calories (Fig. 2C). The locomotor activity was, however, not significantly affected by the diets (Fig. 2D).

To assess whether the rises in blood pressure and heart rate are affected by circadian rhythm, radiotelemetric parameters were examined during the second half of the light period, i.e., 1200–1800 (average of 72 30-s measurements) and during the second half of the dark period, i.e., 2400–0600. Pilot experiments had shown that a 6-h instead of a 12-h period avoided undefined influences caused by changing food and drinking water after 1900. Furthermore, blood pressure and heart rate values were examined for which the locomotor activity was within a specified range. Values were used for averaging only when the locomotor activity was either >80 (arbitrary units) or zero during the 30-s monitoring interval (Fig. 3). An activity >80 corresponded to movement of the rat during the 30-s monitoring interval. Zero locomotor activity was equivalent to no detectable movement of the rat. During the dark period, 33 ± 10% of all values were associated with an activity >80, whereas in the light period, 52 ± 9% of all values were associated with zero activity.

After the 26% sugar (sucrose) calorie diet was fed, systolic blood pressure increased (P < 0.05) compared with that following the 20% fat calorie diet irrespective of day-night cycle (Fig. 3). Systolic blood pressure also increased (P < 0.05) when locomotor activity was absent during the light period (Fig. 3). An increased (P < 0.05) blood pressure was also observed when the locomotor activity was high (>80 arbitrary units) during the dark period. The systolic blood pressure of the sucrose-fed rats during the light period in the absence of locomotor activity was comparable to that of active rats fed only the fat diet (Fig. 3).

Diastolic blood pressure was increased (P < 0.05) after the 40% sugar (sucrose) diet was fed in the dark and light period irrespective of locomotor activity (data not shown). Exchanging sucrose for fructose resulted in an increased (P < 0.05) diastolic blood pressure in the dark period irrespective of locomotor activity and in the light period when locomotor activity was zero (data not shown). When the 26% and 40% sugar (sucrose) diets were fed, heart rate increased (P < 0.05) irrespective of day-night cycle and locomotor activity (data not shown). The fructose feeding increased (P < 0.05) heart rate except during the light period in the absence of locomotor activity (data not shown).

To assess whether the diet-induced rises in blood pressure and heart rate were reversible, rats were fed a regular chow for 2 wk subsequent to the 47% fat-40% sugar (fructose) diet. Both blood pressure and heart rate returned to values observed during the initial feeding of the regular chow (data not shown). Although body weight increased (P < 0.05) during the feeding of the 26% and 40% sugar (sucrose) diets, it did not reach statistical significance after the 40% sugar (fructose) diet was fed for 2 wk (Table 1). Serum parameters were examined in the five yoked rats housed together with the pressure transmitter-implanted rats 2 wk after the fructose diet was fed. Serum glucose and insulin did not differ significantly after the fructose feeding, but the serum triglyceride concentration was increased (P < 0.05) during the feeding of the 26% and 40% sugar (sucrose) diets (Table 1). The proportion of myosin isozyme V1 of left ventricles was also increased (P < 0.05), and the myosin V3 proportion was correspondingly reduced (Table 1).

Fig. 1. Caloric intake and body weight of rats fed various fat/sugar diets. A: total caloric intake (●), intake of fat calories (coconut fat; ♦), and intake of sugar calories (sucrose/fructose; ◆) of experimental rats and total caloric intake of rats fed a regular chow (Δ). Caloric intake was calculated from food intake and caloric content of diets. B: body weight of 10 rats (5 with implanted radiotelemetry pressure transducers and 5 yoked rats kept in same cages) fed experimental diets (●) and 8 rats fed a regular chow (Δ). *P < 0.05 vs. rats fed a regular chow using unpaired 2-sided Student’s t-test (adjusted for unequal variances). Fat percentage values represent fat calories that are 20% (8% wt/wt coconut fat), 35% (16% wt/wt coconut fat), and 47% (24% wt/wt coconut fat) of total calories; 5 percentage values represent sugar calories that are 13% (16% wt/wt sucrose), 26% (32% wt/wt sucrose), and 40% (50% wt/wt sucrose) of total calories; F percentage values represent sugar calories that are 40% (50% wt/wt fructose) of total calories. Sucrose and fructose diets also contained 47% fat calories (24% wt/wt coconut fat).
experiments, the fructose was not dissolved before being mixed with the powdered chow. This diet preparation resulted in a reduced food intake, whereby body weight gain did not occur (Table 2). Blood T3 concentration was raised (P < 0.05) by 99%, which was associated with a marked increase in the proportion of myosin V1 (Table 2). To assess whether the increased myosin V1 proportion can solely be attributed to T3 influences, rats fed the 12% lard-66% fructose diet were treated with the insulin-sensitizing drug BM-13907 (50 mg/kg per day). Although the drug did not reduce serum T3, it lowered (P < 0.05) the proportion of myosin V1 (Table 2).

Table 1. Serum parameters and ventricular myosin isozymes after a 24% coconut fat-50% fructose (wt/wt) diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Regular Chow</th>
<th>47% Fat-40% Sugar Diet [24% Coconut Fat-50% Fructose (wt/wt)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>439 ± 47</td>
<td>458 ± 31</td>
</tr>
<tr>
<td>Left ventricular weight, mg</td>
<td>850 ± 98</td>
<td>873 ± 109</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>207 ± 31</td>
<td>210 ± 36</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>46 ± 13</td>
<td>38 ± 9</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>102 ± 38</td>
<td>304 ± 185*</td>
</tr>
<tr>
<td>T3, ng/dL</td>
<td>86 ± 13</td>
<td>115 ± 17*</td>
</tr>
<tr>
<td>Myosin V1, %</td>
<td>38.2 ± 8.2</td>
<td>55.8 ± 11.3*</td>
</tr>
<tr>
<td>Myosin V2, %</td>
<td>29.0 ± 8.0</td>
<td>27.8 ± 9.0</td>
</tr>
<tr>
<td>Myosin V3, %</td>
<td>32.8 ± 7.8</td>
<td>16.4 ± 7.8*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = n of rats. Rats of diet group were killed 2 wk after having been fed a 47% fat-40% sugar (24% coconut fat-50% fructose (wt/wt)) diet (yoked rats without implanted pressure transmitters). T3, triiodothyronine; V1-3, myosin isozymes. *P < 0.05 vs. rats fed a regular chow by unpaired 2-sided Student’s t-test.
Table 2. Serum parameters and ventricular myosin isozymes after a 12% lard-66% fructose (wt/wt) diet

<table>
<thead>
<tr>
<th>n</th>
<th>12% Lard</th>
<th>12% Lard-66% Fructose</th>
<th>12% Lard-66% Fructose + BM-13907</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>404±10</td>
<td>267±9*</td>
<td>264±10*</td>
</tr>
<tr>
<td>Left ventricular weight, mg</td>
<td>823±30</td>
<td>604±32*</td>
<td>626±30*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>165±15</td>
<td>216±60*</td>
<td>182±19</td>
</tr>
<tr>
<td>T₃, ng/dl</td>
<td>77±9</td>
<td>153±17*</td>
<td>155±22*</td>
</tr>
<tr>
<td>Myosin V₁, %</td>
<td>48.9±5.4</td>
<td>82.1±7.0*</td>
<td>74.4±9.0†</td>
</tr>
<tr>
<td>Myosin V₂, %</td>
<td>29.6±1.8</td>
<td>14.0±4.8*</td>
<td>19.0±6.0†</td>
</tr>
<tr>
<td>Myosin V₃, %</td>
<td>21.5±4.1</td>
<td>3.9±2.4*</td>
<td>6.6±3.1*</td>
</tr>
</tbody>
</table>

The initial body weight was 274±11 g, demonstrating lack of weight gain in the 12% lard-66% fructose fed rats. The dose of 50 mg/kg per day BM-13907 (in the powdered chow) was maintained by monitoring the daily food intake. *P < 0.05 vs. rats fed a 12% lard diet; †P < 0.05 vs. untreated rats fed a 12% lard-66% fructose diet.

DISCUSSION

The aim of the present study was to examine macronutrient influences on blood pressure, heart rate, and locomotor activity of conscious, unrestrained rats. Experimental diets were chosen by taking into account recent trends in human dietary intake. In North America, fat intake has been ranked as 36-41% of total caloric intake (10, 12). In Germany, a fat intake of 40% has been reported (3). The high fat intake represents a phenomenon that has been observed in Westernized societies only during the last few decades (10). The majority of fat is derived from animal sources, which are rich in saturated fatty acids. In the present experimental diets, the caloric intake from fat ranged from 20% to 47% of total calories.

The intake of monosaccharides and disaccharides in the U.S. was estimated as 21% of total calories, 4% of which are derived from fructose, 9% from sucrose, and 5% from sugars in corn syrup (10). For young children in Ontario, Canada, an average of 27% of their dietary energy was estimated to arise from free sugars, namely, 15% from sucrose, 5.2% from lactose, 2.6% from fructose, 2.4% from glucose, and 2.2% from others (2). In the present study, the caloric intake from sucrose or fructose ranged from 13% to 40% of total calories. The diet protocol started with fat feeding, with subsequent sucrose addition to the 47% fat diet. In pilot experiments we have shown that sucrose feeding on its own does not induce a body weight gain (unpublished observations). Because fructose feeding has been examined in detail (14), sucrose was replaced with fructose in the final part of the feeding protocol.

The present study demonstrates that a short-term feeding of diets with an increasing amount of saturated fat and thus caloric content did not significantly raise body weight in rats. The addition of sucrose to the fat diet, however, induced a significant body weight gain. Although the underlying mechanism remains unclear, this observation is in accordance with the finding that overweight in humans appears to be linked mainly with diets rich in fat and mono- or disaccharides (3, 10). The diet-induced rise in body weight was associated with an increased blood pressure (systolic maximum +13 mmHg, diastolic maximum +4 mmHg, mean maximum +7 mmHg) and heart rate (maximum +50 beats/min).

It is a novel finding that the increases in blood pressure and heart rate were affected by neither the day-night cycle nor locomotor activity. As a consequence, the systolic blood pressure of sleeping overfed rats was similar to that of normally fed awake rats. Furthermore, the increased caloric intake was not associated with a higher locomotor activity. Thus the cardiovascular parameters were excessively stimulated when referred to physical activity. The present study also demonstrates that hypercaloric effects on systolic blood pressure first become apparent in the dark period, which is associated with a raised sympathetic activity. In this respect it should be mentioned that the antihypertensive drug moxonidine, which reduces sympathetic outflow of the brain, counteracted a diet-induced rise in blood pressure also mainly during the dark period (20).

Because both heart rate and blood pressure were increased during hypercaloric nutrition, one can conclude that heart work done (mean arterial pressure × heart rate) was also increased. Although not determined in the present study, it can be speculated that a raised sympathetic activity accounts at least partially for the observed increases in blood pressure and heart rate. Evidence in favor of a raised sympathetic activity is the finding that the proportion of myosin isoform V₁ was increased in left ventricles. Myosin V₁ was increased by 18 percentage points, whereas injections of the β₂-adrenoceptor agonist dobutamine increased myosin V₁ by 20 percentage points (17). The shift in myosin isoforms is opposite to that typically observed in hearts with a pathological pressure overload and resembles that of swim-exercised rats (17). Because T₃ was increased in rats fed a 47% fat-40% sugar (fructose) diet, thyroid hormone could contribute to the altered myosin expression.

To further characterize the possible influence of thyroid hormone on an altered myosin as a result of fructose feeding, rats were fed a 12% lard-66% fructose (wt/wt) diet for 7 wk (14). Because the fructose was mixed as a powder with the chow, food intake was reduced and no weight gain was observed. The proportion of myosin V₁ was, however, markedly increased (33 percentage points). Thus a high fructose intake can increase myosin V₁ independently of body weight gain. To examine whether the increased myosin V₁ proportion is possibly associated with the characteristic marked insulin resistance of 66% fructose-fed rats (14), rats were treated with the insulin-sensitizing drug BM-13907 (19). Whereas the serum glucose concentration was increased (P < 0.05) in untreated rats, it was not significantly increased in treated rats. In addition, the proportion of myosin V₁ was reduced (P < 0.05) in treated rats but did not reach the level of that in rats fed 12% lard. Because the serum T₃ concentration was not altered, it can be concluded that the myosin expres-
sion was affected not only by thyroid hormone but also by catecholamines (17) or metabolic influences (19).

It should be pointed out that the rise in systolic blood pressure did not require previous feeding of 60–70% (wt/wt) sugar diets when nontelemetric methods for monitoring blood pressure were used. Thus a 60% (wt/wt) sucrose feeding increased systolic blood pressure and raised sympathetic activity (8). A blood pressure increase and hyperinsulinemia have also been observed after a 12% lard-66% fructose diet was fed (14). In the present study, exchanging sucrose for fructose in the 24% fat-50% sucrose (wt/wt) diet did not induce a greater rise in blood pressure or heart rate. Although the present protocol did not permit the monitoring of serum parameters during the period of 16% and 32% sucrose (wt/wt) feeding, it can be deduced from our previous study (Ref. 20; unpublished data) that moderate hyperinsulinemia had occurred. It was also shown by euglycemic hyperinsulinemic clamps in conscious rats that a 66% sucrose diet was associated with insulin resistance (5a). Evidence in favor of insulin resistance is also the present finding that the triglyceride concentration was increased after the 24% fat-50% fructose (wt/wt) diet was fed.

In rats fed a regular chow, an increase in locomotor activity was associated with a rise in heart rate as well as systolic and diastolic blood pressure (not shown). Furthermore, a close correlation was observed between systolic and diastolic blood pressure. Because blood pressure and heart rate were increased even when the locomotor activity was unchanged in rats fed a hypercaloric diet, it can be concluded that a hypercaloric nutrition induces increased cardiovascular parameters that are in excess when referred to locomotor activity. The rises in blood pressure and heart rate can be reversed after the hypercaloric diet is exchanged for a regular rat chow. Both the parallel rises in blood pressure and heart rate as well as their reversibility are indicative of a state termed hyperkinetic borderline hypertension in humans. This hyperkinetic hypertension was observed in ~30% of patients with borderline hypertension, whereby these patients eventually developed established hypertension (6, 7). On the basis of the present dietary interventions, it is proposed that an excessive caloric intake represents a crucial determinant of hyperkinetic hypertension.

The assistance of Karin Rupp is greatly appreciated.

This study was supported by the German Research Foundation (DFG Ru 245/7-1) and the Alfred-Teufel-Stiftung.

Address for reprint requests and other correspondence: H. Rupp, Molecular Cardiology Laboratory, Dept. of Internal Medicine and Cardiology, Philipps Univ. of Marburg, Karl-von-Frisch-Str. 1, 35033 Marburg, Germany (E-mail: Rupp@mailer.uni-marburg.de).

Received 1 September 1998; accepted in final form 31 May 1999.

REFERENCES


