CLINICAL STUDY

Weight gain by hyperalimentation elevates C-reactive protein levels but does not affect circulating levels of adiponectin or resistin in healthy subjects

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Abstract

Objective: Increase of resistin and/or reduction of adiponectin have been implicated in the development of insulin resistance following weight gain. We aimed to study this prospectively in humans.

Design: Prospective and interventional with parallel control group.

Methods: Twelve healthy men and six healthy women (age 26 ± 6.6 years) and an age-matched control group were recruited. Subjects in the intervention group aimed for a bodyweight increase of 5–15% by doubling the baseline caloric intake by eating at least two fast food-based meals a day in combination with adoption of a sedentary lifestyle for 4 weeks.

Results: Bodyweight increased from 67.6 ± 9.1 to 74.0 ± 11 kg, $P < 0.001$, by the intervention. Insulin levels increased (before: 27.4 ± 12 pmol/l, after: 53.0 ± 22 pmol/l, $P = 0.004$), while plasma levels of adiponectin (before: 5038 ± 3736 ng/ml, after: 6739 ± 7949 ng/ml, $P = 0.18$) and resistin (before: 21.8 ± 19 ng/ml, after: 14.4 ± 6.8 ng/ml, $P = 0.074$) remained unchanged by the weight gain and were similar as in controls. On the other hand, leptin levels increased about threefold following the intervention (before: 5.7 ± 7.4 , after: 16 ± 20 ng/ml, $P = 0.008$), and also the inflammatory marker C-reactive protein (CRP) increased from 0.34 ± 0.44 to 0.71 ± 0.87 mg/l, $P = 0.03$, when two outliers > 10 mg/l were disregarded.

Conclusions: Hyperalimentation reduces insulin sensitivity when weight gain of 9% was combined with reduction of exercise. However, the levels of resistin and adiponectin were unaffected by the intervention, while CRP levels increased within this short time period suggesting that low-grade inflammation can occur early in the process of developing a metabolic syndrome.

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Introduction

The worldwide increase in the prevalence of obesity is expected to relate to a marked rise in the number of patients with type 2 diabetes. However, the exact mechanisms behind the development of the insulin resistance that is a consequence of obesity are not known in humans. A lot of attention has recently been paid to the secretory properties of adipocytes and of the fat tissue. Secreted hormones from the fat tissue are collectively denominated adipokines, and according to experimental studies several key candidates among these proteins are capable of affecting both glucose metabolism and energy balance (1). Leptin is secreted by fat cells and acts on the CN where specific receptors are expressed in hypothalamic neurons that can regulate appetite. Circulating leptin levels in humans

are proportional to adipose tissue mass and are also associated with the presence of cardiovascular disease (2, 3). Indeed, several atherogenic properties of leptin have been described *in vitro* (3). The adipokine adiponectin is also secreted by adipocytes and is found in the circulation in several isoforms. Adiponectin might protect against the development of type 2 diabetes (4) and thus seems to counteract insulin resistance, and in many studies adiponectin levels are elevated by physical exercise in humans (5). However, it is mainly the heavy molecular weight (HMW) isoform that has been linked with insulin-sensitizing effects in humans (6, 7). While leptin and adiponectin are produced by the fat cells, the 12 kDa peptide resistin is derived from macrophages in the fat tissue (8), and the levels of circulating resistin are associated with the prevalence of insulin resistance in humans in several (9, 10), but not

all (11), cross-sectional studies. It has been proposed that resistin acts as a mediator of the chronic inflammatory process that has been the focus of much research in obesity and insulin resistance (12, 13). C-reactive protein (CRP) is an acute-phase reactant and is considered a classic marker of inflammation. CRP levels within the range detected with high-sensitivity (hs) assays, levels <1, 1–3 and >3 mg/l, correspond to low-, moderate-, and high-risk groups for future cardiovascular events, while higher levels are observed during acute inflammation such as in infectious disease (14).

The clinical correlates of levels of adipokines are corroborated by the finding that the circulating levels of resistin in humans are lowered by treatment with the anti-diabetic insulin-sensitizing PPAR γ agonist drugs rosiglitazone and pioglitazone, while such treatment concomitantly increases the levels of adiponectin (15–17).

The great majority of prospective interventional studies of the development of insulin resistance are based on *in vitro* experiments or on studies in animals. In contrast, little is known about the early phases in the development of insulin resistance in humans. This is likely a consequence of that it is cumbersome, and even unpleasant, to participate in trials in which the participants are expected to gain weight to an extent large enough to affect clinical markers of insulin sensitivity. Consequently, most studies on insulin resistance in humans are observational, or the effects are supposed to be possible to derive from trials in which already obese subjects are subjected to weight reduction by different procedures or are given pharmacological substances. In contrast, we performed a prospective study of fast food-based hyperalimentation in healthy subjects who were asked to double the regular calorie intake and also to abandon physical exercise for 4 weeks. The aims of this analysis were to prospectively investigate the development of reduced insulin sensitivity and markers of the metabolic syndrome, in relation to changes in the circulating levels of leptin, resistin, and HMW adiponectin, and also to study this in relation to the presumed increase in intra-abdominal obesity as determined by magnetic resonance imaging (MRI).

Methods

Intervention group

We recruited 12 males and 6 females as volunteers for the intervention arm of the study. Age- and gender-matched subjects for the control group were recruited in parallel. The design was thus prospective and interventional with a parallel control group. The participants of the intervention arm had to accept an increase in body weight of 5–15% and were subsequently asked to eat at least two fast food-based meals a day, preferably

at well-known fast food restaurants such as McDonald's and Burger King. The results of the intervention on liver enzymes and on gender differences in changes in body composition have been published earlier (18, 19). The food expenses were reimbursed consecutively, and information based on the food receipts were also used for estimation of the actual food composition and calorie intake. Physical activity was not to exceed 5000 steps per day. The maximal weight gain was set to 15%, and the subjects were asked to terminate the study as soon as possible after re-performing the same study investigations as were done at baseline if this level of body weight increase was reached within the 4-week period. All the participants were free from significant diseases as judged by medical check-up and history at recruitment.

The subjects in the intervention group were contacted and given advice by professional dietitians, by weekly meetings or by phone, during the study. The aims of these advices were to affect the calorie intake to correspond to a doubling of the regular calorie requirement that they had before entering the trial period. If the subject was not able to ingest the hamburger-based diet, it was changed to whatever food the participant could presently accept with the main aim to achieve the calculated calorie intake and also to accomplish a food composition rich in animal protein and fat. The exact composition of the diet, e.g. data on unsaturated or saturated fat, saccharides and complex carbohydrates, was calculated from reports done 3 days before the study and another two 3-day periods: one at the end of the first and one during the third study week (or a week earlier in one subject who ended the trial after just 2 weeks).

Blood for routine laboratory tests was drawn in the fasting state at baseline, i.e. before starting on the extra calorie intake, after 2 weeks on the fast food-based diet, and at the end of the study, i.e. either at the end of fourth week or earlier if prematurely terminated. Since very few studies that deliberately aimed to reduce insulin sensitivity have been performed earlier, blood was also drawn in the non-fasting state at the end of the first and the third study weeks, as a precaution, to monitor the changes in serum liver enzyme levels and non-fasting lipid levels.

HMW adiponectin, resistin, and leptin were measured using ELISA kits (Linco Research, St Charles, MO, USA) in duplicates. The intra-assay coefficient of variation (CV) for leptin was 2.4% for low and 3.5% for high controls, and the corresponding total assay CV were 5.9 and 4.1% respectively. The methodological error for leptin was 10.3% (CV). The intra-assay CV for HMW adiponectin was 5.9% for low and 11.4% for high controls, and the total assay CV were 9.0 and 12.0% respectively. The methodological error for HMW adiponectin was 7.9%. The intra-assay CV for resistin was 6.1% for low and 10% for high controls, and the total assay CV were 24 and 11% respectively. The methodological error for the determination of resistin levels was

9.3%. Serum insulin was assayed using immunoassay methods (AutoDelfia, Perkin Elmer, Linköping, Sweden). Total cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglycerides were determined by colorimetric analyses (Siemens, Liederbach, Germany), and low-density lipoprotein cholesterol was calculated according to Friedewald ((total cholesterol – HDL-cholesterol) – (0.456 × total triglyceride concentration)). Glucose was determined by the hexokinase method (Siemens), and hs-CRP was determined by spectrophotometry (Wide range CRP, Siemens). Homeostasis model assessment (HOMA) index of insulin resistance was calculated as glucose concentration × insulin concentration / 22.5 (20), while the quantitative insulin sensitivity check index (QUICKI) was calculated as $1/(\log(\text{insulin concentration}) + \log(\text{glucose concentration}))$ (21).

The subjects were subjected to dual energy X-ray absorptiometry (DEXA: Hologic 4500, Hologic, Waltham, MA, USA) for analysis of body composition. The technique for the measurement of basal metabolic rate has been described earlier (22) and was based on analysis of CO₂ production and O₂ consumption with Delta Trac equipment (SensorMedics, Yorba Linda, CA, USA). The measurement of intra-abdominal and subcutaneous fat volumes by MRI has also been described in detail earlier (19).

All anthropometric measurements were made by two research nurses. The control group performed the laboratory investigations, the measurement of basal metabolism, and the anthropometric measurements at baseline and after 4 weeks.

Statistical analysis

Statistical calculations were done with PASW 18.0 software (SPSS Inc., Chicago, IL, USA). Linear correlations were calculated as stated in the text. Comparisons within and between groups were done with Student's paired and unpaired two-tailed *t*-test or as stated in the Results section. Mean values and s.d.s are given, unless otherwise stated. Statistical significance was considered at the 5% level ($P \leq 0.05$). Data on HMW adiponectin were missing in two controls at baseline and in one subject in each of the two groups at the end of the study due to technical reasons, and data on hs-CRP were missing in two subjects of the intervention group at the end of the study. Since the detection limit of hs-CRP was 0.30 mg/l, levels below the detection limit were set at 0.15 mg/l in the statistical analyses and in the figure.

Ethics

The study was approved by the Regional Ethics Committee of Linköping and performed in accordance with the Declaration of Helsinki. A written informed consent was obtained from all the participating subjects.

Results

All the subjects in the intervention group except one were students. Seventeen of the eighteen participants met the goal to increase 5–15% body weight by the intervention, while one participant increased 3.3% in body weight. Four men and one woman reached the maximal 15% increase in body weight. Mean daily calorie intake during the total intervention period increased by $+70 \pm 35\%$ (men $+68 \pm 31\%$ and in women by $+74 \pm 45\%$). There was no statistically significant change in the food intake of macronutrients when comparing the registrations from the first and third weeks, nor did we find any gender differences regarding macronutrient composition of the hyperalimentation. The subject with the steepest weight increase started at 79.8 kg and reached 91.9 kg already after 2 weeks ($+15\%$), and thus terminated the study early. One male participant developed alanine aminotransferase (ALT) level of 447 U/l (7.6 $\mu\text{kat/l}$) during the third week (18) and was asked to reduce his calorie intake for reasons of medical safety at this time point.

Table 1 shows baseline anthropometric and laboratory data of all the participants and the effects of the intervention. Data were similar in the control and subjects of the intervention group at baseline and were unchanged in the controls during the 4-week observation period (Table 1). The subjects of the intervention group displayed a pronounced increase in body weight and a concomitant increase in fasting insulin levels (Table 1). When analyzed according to gender, there was almost a twofold increase in insulin levels in men (before: 27.4 ± 12 pmol/l, after: 53.0 ± 22 pmol/l, $P=0.004$) with no statistically significant changes in the women (before: 35.0 ± 16 pmol/l, after: 42.5 ± 20 pmol/l, $P=0.17$) (19). HOMA index of insulin resistance (20) increased, and QUICKI of insulin sensitivity (21) was lowered in the intervention group but unchanged in the controls (Table 1).

Mean circulating plasma resistin and HMW adiponectin were similar in the controls and in the subjects of the intervention group before and at the end of the study and remained statistically unchanged in the controls (controls: HMW adiponectin before: 3244 ± 3155 ng/ml, after: 2709 ± 2583 ng/ml, $P=0.28$ and resistin before: 18.5 ± 17 ng/ml, after: 13.6 ± 3.4 ng/ml, $P=0.24$; intervention group: HMW adiponectin before: 5038 ± 3736 ng/ml, after: 6739 ± 7949 ng/ml, $P=0.18$ and resistin before: 21.8 ± 19 ng/ml, after: 14.4 ± 6.8 ng/ml, $P=0.074$, no statistical significant differences compared with the control group at any time point). Figure 1a (resistin) and b (HMW adiponectin) show individual changes in the subjects of the intervention group during the weight gain. There were also no statistically significant changes in the levels of resistin or HMW adiponectin when data were analyzed in men and women separately (not shown). Leptin, on the other hand, increased on average

Table 1 Anthropometrics, basal metabolic rate, and fasting insulin of the control group (C) in comparison with the intervention group (I). There were no differences in any of the parameters between the groups at baseline and no statistically significant changes within the control group.

Variable	Baseline		After 4 weeks		P value (C vs I)	P value (Baseline vs After 4 weeks) in the I group
	C	I	C	I		
Age (years)	25±3.5	27±6.6				
Sex (male/female)	12/6	12/6			Matched	
Weight (kg)	69.7±8.4	67.6±9.1	69.7±8.7	74.0±11	0.2	<0.0001
Body mass index (kg/m ²)	22.2±2.1	21.9±1.9	22.2±2.2	23.9±2.2	0.02	<0.0001
Abdominal sagittal diameter (cm)	17.8±1.3	18.4±1.7	17.8±1.4	20.4±1.6	<0.0001	<0.0001
Waist circumference (cm)	75.5±5.8	76.4±6.4	75.4±6.0	83.1±7.9	0.002	<0.0001
Hip circumference (cm)	89.0±6.9	86.5±7.1	89.8±6.1	90.4±8.5	0.8	0.03
Systolic BP (mmHg)	118±6.7	112±12	118±8.5	116±16.8	0.06	0.18
Diastolic BP (mmHg)	73±4.8	67±7.2	72±7.8	69±4.8	0.8	0.43
Basal metabolic rate (kcal/24 h)	1700±243	1614±276	1712±262	1813±327	0.3	0.001
Total fat mass by DEXA (kg)	N/A	12.7±5.7	N/A	16.4±5.5	N/A	<0.0001
Intra-abdominal fat mass by MRI (l)	N/A	1.28±0.78	N/A	1.73±0.81	N/A	<0.0001
Fasting insulin (pmol/l)	37.8±24	29.9±14	37.9±19	49.5±21	0.06	0.002
Fasting glucose (mmol/l)	4.81±0.37	4.74±0.35	4.88±0.28	5.07±0.49	0.17	0.013
HOMA	1.2±0.85	0.89±0.42	1.0±0.54	1.6±0.83	0.02	0.002
QUICKI	0.383±0.027	0.403±0.041	0.394±0.044	0.366±0.033	0.03	0.001
Total cholesterol (mmol/l)	4.0±0.67	4.1±0.62	4.1±0.77	4.5±0.61	0.059	0.002
LDL-cholesterol (mmol/l)	2.3±0.55	2.3±0.54	2.4±0.54	2.5±0.60	0.54	0.018
HDL-cholesterol (mmol/l)	1.3±0.23	1.5±0.41	1.3±0.25	1.8±0.49	0.007	0.057
Triglycerides (mmol/l)	0.92±0.53	0.72±0.21	0.80±0.35	0.75±0.34	0.63	0.86

*Blood pressures in the intervention group were the mean value of recordings made at several of the investigations (only performed in the intervention group), while corresponding measurements in the controls were based on one recording; however, the *P* value refers to comparison of measurements made at the same occasion for both groups.

about threefold in the subjects of the intervention group (before: 5.7 ± 7.4 ng/ml, after: 16 ± 20 ng/ml, $P=0.008$, Fig. 1c), while being similar in both the groups at baseline (control group baseline: 7.0 ± 9.6 , after 4 weeks: 6.3 ± 7.5 ng/ml, $P=0.6$ for change and $P=0.6$ for comparison of baseline levels with the intervention group). The relative increase in leptin was of a similar magnitude, about threefold, in both men (2.3 ± 1.7 after: 5.9 ± 3.7 ng/ml, $P=0.003$) and women (before: 12.5 ± 10 , after: 36.0 ± 27 ng/ml, $P=0.03$). Unpaired comparisons between the intervention group and the control group resulted in a *P* value bordering on statistical significance when comparing leptin levels after 4 weeks between the groups ($P=0.07$).

At baseline, HOMA (index of insulin resistance) and QUICKI (index of insulin sensitivity) both related to leptin levels (HOMA: $r=0.55$, $P=0.001$; QUICKI: $r=-0.35$, $P=0.04$) in the total material, but only QUICKI related to the levels of HMW adiponectin (HOMA: $r=-0.27$, $P=0.14$; QUICKI: $r=0.45$, $P=0.01$). There were no statistically significant correlations between HOMA and QUICKI to the levels of resistin (all $P>0.3$) at baseline. There were also no statistically significant correlations between the concentrations of the three adipokines (all $P>0.14$) at baseline.

In this group of healthy non-obese subjects, only circulating leptin of the three adipokines studied related to total amount of adipose tissue by DEXA ($r=0.79$, $P<0.001$) and amount of adipose tissue by MRI of abdominal region ($r=0.47$, $P=0.049$). When analyzing the changes in adipokines in relation to changes in the adipose tissue measures, again, only changes in the circulating leptin were statistically significant related to the measures of fat mass (ratio of leptin after/before correlated to the corresponding ratio of increase in intra-abdominal fat mass: $r=0.75$, $P<0.0001$ and to amount of increase in total fat tissue ratio by DEXA: $r=0.76$, $P<0.0001$).

Two subjects in the intervention group had CRP above 10 mg/l, one at baseline and another at the study end. When analyzing the data in the remaining group, after removing these two outliers, a statistically significant increase in CRP levels following weight gain was observed (hs-CRP before: 0.34 ± 0.44 mg/l, after: 0.71 ± 0.87 mg/l, $P=0.03$, see also Fig. 1d). In the controls, there was no change in the levels of hs-CRP during the observation period (before: 1.2 ± 2.4 mg/l, after: 0.57 ± 0.81 mg/l, $P=0.24$). Resistin levels were 20.6 ± 20 ng/ml before and 14.5 ± 7.2 ng/ml after the intervention ($P=0.17$), when these two outliers with respect to hs-CRP were excluded. Corresponding values

Figure 1 Circulating fasting levels of resistin (a), adiponectin (b), leptin (c), and hs-CRP (d) before and after aiming to double the calculated regular caloric intake and combining this with adoption of a sedentary behavior for 4 weeks. The change in leptin levels was statistically significant, before: 5.7 ± 7.4 ng/ml and after: 16 ± 20 ng/ml, $P=0.008$ in paired t -test. Observe that in (d), five subjects had an hs-CRP level of 0.15 mg/l both before and after the intervention, and that these subjects thus cannot be individually visualized. Also note that two subjects with an hs-CRP above 10 mg/l were excluded from the graph. Levels of hs-CRP were 0.34 ± 0.44 mg/l before and 0.71 ± 0.87 mg/l after the intervention in these subjects, $P=0.03$ in paired t -test.

for HMW adiponectin were 5085 ± 3782 ng/ml before and 6817 ± 8326 ng/ml after the intervention ($P=0.23$), while the levels of leptin were 5.8 ± 7.8 ng/ml before and 17 ± 22 ng/ml after the intervention ($P=0.013$) after exclusion of the two outliers.

Discussion

Despite successfully reducing insulin sensitivity by combining hyperalimentation with a sedentary behavior, we found no changes in the circulating levels of resistin or HMW adiponectin, even though the weight gain was 9%. On the other hand, the levels of leptin were increased almost threefold by the intervention. The increase in fasting levels of insulin was particularly pronounced in the men, but also when analyzed according to gender, the levels of resistin and HMW adiponectin were statistically unchanged following the weight gain.

Although our study was not numerically large, we know of no other larger studies with participants of both genders that induced weight gain and concomitant increase in fasting insulin of a similar magnitude as in our study. We found it unlikely that low statistical power was the reason for the lack of increase in resistin or decreases of HMW adiponectin since there were no trends for such changes to occur in our study, although we acknowledge the small size of the study as a limitation. Also, the graphical appearance in Fig. 1a and b clearly displays that the individual participants exhibited increases or decreases of resistin and HMW adiponectin during the intervention in a manner seeming to be arbitrary, which suggests that other

factors than weight gain determined the changes in the levels of these hormones. Also when analyzing the changes in intra-abdominal fat tissue by MRI-based quantification of adipose tissue volume, we found no significant relationships with either circulating levels of HMW adiponectin or resistin. However, it should be noted that the lack of correlation in the cross-sectional analysis within the group at baseline was hampered by the small variation in weight in this group of healthy non-obese subjects.

The results of our study thus suggest that increases in resistin and/or decreases of HMW adiponectin are not primary phenomena that are necessary for the earliest steps in the development of reduced insulin sensitivity caused by weight gain and low levels of physical activity. According to our study, such changes are instead likely to be secondary phenomena that still might be important for the long-term changes and manifestations of insulin resistance. Indeed, although re-feeding of patients with anorexia nervosa might not be a model for development of insulin resistance, it has earlier been demonstrated that during the early stage of weight increase, HMW adiponectin levels increase as do markers of reduced insulin sensitivity (23). Also suggestive of a sufficient weight gain for relevant changes in hormone levels to occur in our study was the recent finding that a weight loss of 5–10%, but not of 5%, was sufficient to induce changes in adipokines in severely obese women (24). We have earlier shown that saturated and, in particular, mono-unsaturated fatty acids can stimulate the transcription factor PPARG in primary human fat cells (25), and PPARG stimulation promotes the release of HMW adiponectin (26). Thus, during hyperalimentation it is possible that increase in

fatty acids could induce the release of HMW adiponectin despite weight gain and reduced insulin sensitivity, again; however, these effects might only be present during the short hyperalimentation phase, and not during more permanent obesity. Our study adds to the idea that there is no proof yet that adiponectin actually modulates insulin sensitivity in humans, and that low adiponectin levels may be a consequence of the hyperinsulinemia in insulin resistance as described in a recent review on adiponectin in human metabolic syndrome by Cook *et al.* (27).

Intriguingly, we did find a small but statistically significant increase in hs-CRP levels following the intervention. This should be interpreted with caution, however, since the data relied on comparisons of 14 samples and hs-CRP is a rather unspecific marker of inflammation that can react in response to numerous stimuli, making it difficult to interpret the results on an individual level or even in small groups, such as in our study. The finding of increased levels of hs-CRP following weight gain is, however, corroborated by an observational study in which the spontaneous weight gain during 9 years was associated with an increase in hs-CRP (28), and with cross-sectional findings of elevated levels of hs-CRP in obese and overweight subjects (29). Tam *et al.* (30) also recently demonstrated a significant increase in hs-CRP by overfeeding for 28 days that caused an average weight gain of 2.7 kg. The finding of a small but statistically significant increase in inflammation, hs-CRP, but lack of a corresponding increase in levels of resistin suggests that resistin is not an orchestrator of inflammation in the early stage of insulin resistance, as has been suggested by others (12, 13). Indeed, some quite recent studies even showed an inverse relation between resistin and markers of insulin resistance (11, 31).

In contrast to HMW adiponectin and resistin, the levels of leptin increased quite dramatically in both men and women of the intervention group. Since the increase in fasting insulin and other markers of reduction of insulin sensitivity only occurred to a significant degree in the males, leptin is probably not a key player to be causative in terms of mediating insulin resistance. Indeed, leptin might even have a protective role against obesity and its consequences since the administration of leptin can increase the basal metabolic rate when administered after weight reduction (32), and s.c. injections of leptin reduces glucose levels in leptin deficiency (33). On the other hand, whether leptin causes inflammation in humans when administered as s.c. injections (34), or not (35), is presently controversial. The effect of leptin resistance in obesity, which has been demonstrated in humans (36), could also not be determined in our study but could have affected the levels of the studied hormones.

In summary, we found no increases in HMW adiponectin or resistin despite reduced insulin sensitivity in 18 subjects that combined weight gain of 9% by

hyperalimentation with reduction of physical activity. However, the levels of hs-CRP did increase following intervention suggesting that low-grade inflammation can indeed occur early in the process of development of the metabolic syndrome. According to our intervention study, the adipokines resistin and HMW adiponectin are unlikely to be the mediators of such primary events in the development of a metabolic syndrome following weight gain. Since the study was of limited size, future studies are called for to re-assess the findings.

Declaration of interest

The funding sources had no impact on the design or performance of the study, and there were no competing interests for any of the authors in relation to this manuscript.

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