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Transient increase in HDL cholesterol during weight gain by hyper-alimentation in healthy subjects

Running title: HDL increase by fast-food

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Abstract

Determination of lipid levels is fundamental in cardiovascular risk assessment. We studied the short term effects of fast food-based hyper-alimentation on lipid levels in healthy subjects. Twelve healthy men and six healthy women with a mean age of 26±6.6 years and an aged-matched control group were recruited for this prospective interventional study. Subjects in the intervention group aimed for a body weight 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 four weeks. This protocol induced a weight gain from 67.6±9.1 kg to 74.0±11 kg ($p<0.001$). A numerical increase in the levels of HDL cholesterol occurred in all subjects during the study and this was apparent already at the first week in 16/18 subjects (mean increase at week one: +22.0±16%, range from –7 to +50%) while the highest level of HDL during the study as compared with baseline values varied from +6% to +58% (mean +31.6±15%). The intake of saturated fat in the early phase of the trial related positively with the HDL cholesterol-increase in the second week ($r=0.53$, $p=0.028$). Although the levels of insulin doubled at week two, the increase in LDL cholesterol was only +12±17% and there was no statistically significant changes in fasting serum triglycerides. We conclude that hyper-alimentation can induce a fast but transient increase in HDL cholesterol that is of clinical interest when estimating cardiovascular risk based on serum lipid levels.

INTRODUCTION

Determination of cholesterol levels is a cornerstone for estimation of cardiovascular risk. Dyslipidemia, i.e. high triglycerides and low HDL cholesterol, is often seen in conjunction with abdominal obesity and poor physical fitness, as in the metabolic syndrome. Life style changes such as weight loss by reduced caloric intake and increased exercise can reverse unfavorable levels of triglycerides and HDL cholesterol. The levels of LDL cholesterol, however, are less obviously linked to obesity and insulin resistance as exemplified by the rather common condition heterozygote familial hypercholesterolemia, which is the consequence of inherited defect in LDL receptor level or activity. Oxidization of the LDL cholesterol is an early step in the atherosclerotic process and oxidized LDL exists in vivo in humans (1). Traditionally a low fat diet has been advocated to avoid cardiovascular disease despite the fact that a change to such diets have not hitherto demonstrated a reduction in the incidence of cardiovascular disease (2). In addition of constituting a large part of the body mass in the form of triglycerides, fatty acids have been shown to have an inherent capacity to affect gene transcription through activation of transcription factors such as those of the peroxisome proliferator beta and gamma (3). Indeed, both unsaturated and saturated fatty acids can induce PPAR gamma receptor activity in primary human fat cells (3). High PPAR gamma receptor activity is linked to reduced dyslipidemia in humans as shown by treatment with thiazolidinediones (rosiglitazone (4) and pioglitazone (5)) that are strong synthetic PPAR gamma agonists that increase HDL cholesterol levels in parallel with the reduction of insulin resistance. Interestingly, low endogenous PPAR gamma receptor activity has been demonstrated in the omental fat tissue in primary human fat cells (6) and it is the amount of such intra abdominal fat that seems to be most relevant for development of the metabolic syndrome (7). The risk for cardiovascular disease related to components of the

metabolic syndrome thus not only depends on hereditary factors and obesity but also on where the excess fat is stored in the abdominal region and potentially also on hormonal effects of fatty acids *per se*.

We performed a study of fast food based hyper-alimentation in healthy subjects who were also required to abandon physical exercise during the study period of four weeks. The aims of the study were to prospectively evaluate the occurrence of presumed signs of insulin resistance and dyslipidemia by the ensuing lack of physical fitness and weight gain compared with matched controls.

METHODS AND PROCEDURES

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 participants of the intervention group 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 body composition has been published earlier (8,9). The food expenses were reimbursed consecutively and the food receipts were also used for estimation of the actual food composition and caloric intake. Physical activity was not to exceed 5000 steps per day. The maximal weight gain was 15% and subjects were asked to terminate the study as soon as possible by re-performing the same study investigations as were done at baseline if this level of body

weight increase was reached within the stipulated four week trial period. The participants were all free from significant diseases as judged by medical check-up and history at recruitment.

Subjects in the intervention group were given advice by professional dieticians, by weekly meetings or by phone, during the study. The advices were individually adjusted to result in an intake corresponding to doubling the present caloric requirement. If the subject was not able or willing to ingest the hamburger-based diet, it was changed to whatever food the participant accepted with the aim to achieve the calculated caloric intake and also, if the study subject still found it acceptable, to accomplish a food composition rich in protein and saturated animal fat. Habitual weekly alcohol consumption was assessed at study entry and all subjects were asked to keep alcohol intake unchanged during the study.

The exact composition of the diet, for example data on unsaturated or saturated fat, saccharides or complex carbohydrates, was determined based on reports from three days before the study and another two three day periods at the end of the first or third weeks (or a week earlier in the one subject that ended the trial after two weeks).

Blood for laboratory tests was drawn in the fasting state at baseline, i.e. before starting on the extra caloric intake, after two 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 changes in serum liver enzyme levels and non fasting lipid levels. Circulating oxidized LDL cholesterol was measured with an ELISA-kit (Immundiagnostik AG, Bensheim, Germany).

The intra-assay variation was 4.5% (mean value 38.6 ng/mL, n=29) and the total-assay variation was 5.2 %. The methodological error of the ELISA was 6.3 % (coefficient of variation) as calculated from duplicate measurements in 107 patient samples. Plasma LDL-cholesterol concentration was calculated with the use of Friedewald´s formula, the methods for analysis of the other samples have been published (8,9).

The subjects were subjected to dual energy x-ray absorbimetry (Hologic 4500, Hologic, Waltham, MA, USA), for analysis of body composition. All anthropometric measurements were done by two research nurses. The control group performed the laboratory investigations and anthropometric measurements at baseline and after 4 weeks.

Statistics

Statistical calculations were done with SPSS 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 2-tailed t-test or as stated in the results section. Mean (SD) is given unless otherwise stated. Statistical significance was considered at the 5% level ($p \leq 0.05$).

Ethics

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

RESULTS

Table 1 shows baseline anthropometric and laboratory data of all the participants, and the effects of the intervention on a weekly basis of subjects in the intervention group (note that laboratory analyses were done on blood in the non-fasting state in weeks 1 and 3). All subjects in the intervention group except one were students. Seventeen of the 18 participants met the goal to increase 5-15% body weight by the intervention while one participant merely increased 3.3% in body weight. Mean daily caloric intake of the total intervention period increased +70 ± 35 % (men +68 ± 31 % women +74 ± 45 %). Two men and two women managed to consume a mean daily caloric intake > + 90% of basal during the total duration of the study. 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 hyper-alimentation. Four men and one woman in the intervention group reached 15% increase in body weight. The subject with the most steep body weight increase started at a weight of 79.8 kg and reached 91.9 kg already after two weeks (+15%), and thus terminated the hyper-alimentation. One male participant developed an ALT level of 447 U/l (7.6 μkat/l) during the third week (8), and thus was asked to reduce his caloric intake at this time point.

A numerical increase in the levels of HDL cholesterol occurred in all subjects during the study as is graphically displayed in figure 1. In most cases the increase was apparent already at week 1 as is also seen in the figure (mean increase at week 1: + 22.0 ± 16%, range from – 7 to +50%) while the highest level of HDL during the study as compared with baseline values varied from + 6% to

Table 1. Anthropometric and laboratory data in the controls and in subjects of the intervention group before, during and at the end of the hyper-alimentation. Figures are means (SD). The reduced numbers of observations of LDL levels in week one and three were due to increased triglyceride levels that rendered Friedewalds formula unusable. * = p <0.05 as compared with baseline within intervention group, # = p <0.05 in unpaired t-test between controls and intervention group at the same time point. Although triglycerides were analyzed at weeks one and three in subjects of the intervention group these data are not presented since these non-fasting results not fairly can be compared with the other fasting samples.

Variable	Group	Baseline	Week 1 (non fasting)	Week 2	Week 3 (non fasting)	Week 4 (or end of diet in the intervention group)
Age (yr)	Int. group controls	27 (6.6) 25 (3.5)				
Gender (M/F)	Int. group controls	12/6 12/6				
Weight (kg)	Int. group controls	67.6 (9.1) 69.7 (8.4)	70.6 (10)* ND	71.8 (10)* ND	72.0 (9.6)* ND	74.0 (11)* 69.7 (8.7)
Sagittal abdominal diameter (cm)	Int. group controls	18.4 (1.7) 17.8 (1.3)	ND	19.4 (1.8)* ND	ND	20.4 (1.6)* 17.8 (1.4)#
Waist circumference (cm)	Int. group controls	76.4 (6.4) 75.5 (5.8)	ND	80.1 (6.4)* ND	ND	83.1 (7.9)* 75.4 (6.0)#
Hip circumference (cm)	Int. group controls	86.5 (7.1) 89.0 (6.9)	ND	88.1 (6.9) ND	ND	90.4 (8.5)* 89.8 (6.1)
Fs-insulin (pmol/l)	Int. group controls	29.9 (14) 37.8 (24)	ND	59 (35)* ND	ND	49.5 (21)* 36.1 (20)
Total cholesterol (mmol/l)	Int. group controls	4.1 (0.62) 4.0 (0.67)	4.8 (0.58)* ND	4.8 (0.63)* ND	5.0 (0.85)* ND	4.5 (0.61)* 4.1 (0.77)
LDL cholesterol (mmol/l)	Int. group controls	2.3 (0.54) 2.3 (0.55)	2.3 (0.54)*[a] ND	2.5 (0.56)* ND	2.6 (0.80) [b] ND	2.5 (0.60)* 2.4 (0.54)
HDL cholesterol (mmol/l)	Int. group controls	1.5 (0.41) 1.3 (0.23)	1.8 (0.46)* ND	1.9 (0.49)* ND	1.8 (0.49)* ND	1.6 (0.45) 1.3 (0.25)#
Triglycerides (mmol/l)	Int. group controls	0.72 (0.21) 0.92 (0.53)	Not fasted ND	1.0 (0.58) ND	Not fasted ND	0.75 (0.34) 0.80 (0.35)
ApoB (g/l)	Int. group controls	0.73 (0.16) 0.77 (0.17) [a]	ND	0.87 (0.21)* ND	ND	0.82 (0.22) 0.78 (0.18)
ApoA1 (g/l)	Int. group controls	1.55 (0.40) 1.64 (0.22) [a]	ND	1.88 (0.40)* ND	ND	1.75 (0.37)* 1.5 (0.22)#
Oxidized LDL (ng/ml)	Int. group controls	277 (242) 234 (139)	ND	ND	ND	256 (222) 304 (267) [b]
Body fat (%)	Int. group controls	20.1 (9.8) ND	ND	ND	ND	23.8 (8.6)* ND

Notes, a: n=16, b: n= 15

+ 58% (mean level of +31.6 ± 15%). There was no change in levels of oxidized LDL cholesterol in the intervention group (Table 1) and serum lipids and lipoprotein levels remained unchanged during the study period in the control group. There were no differences in lipid levels between the

groups at baseline but at the end of the study the intervention group showed higher HDL cholesterol and Apo A1 levels than controls while LDL cholesterol levels and Apo B were similar in both groups.

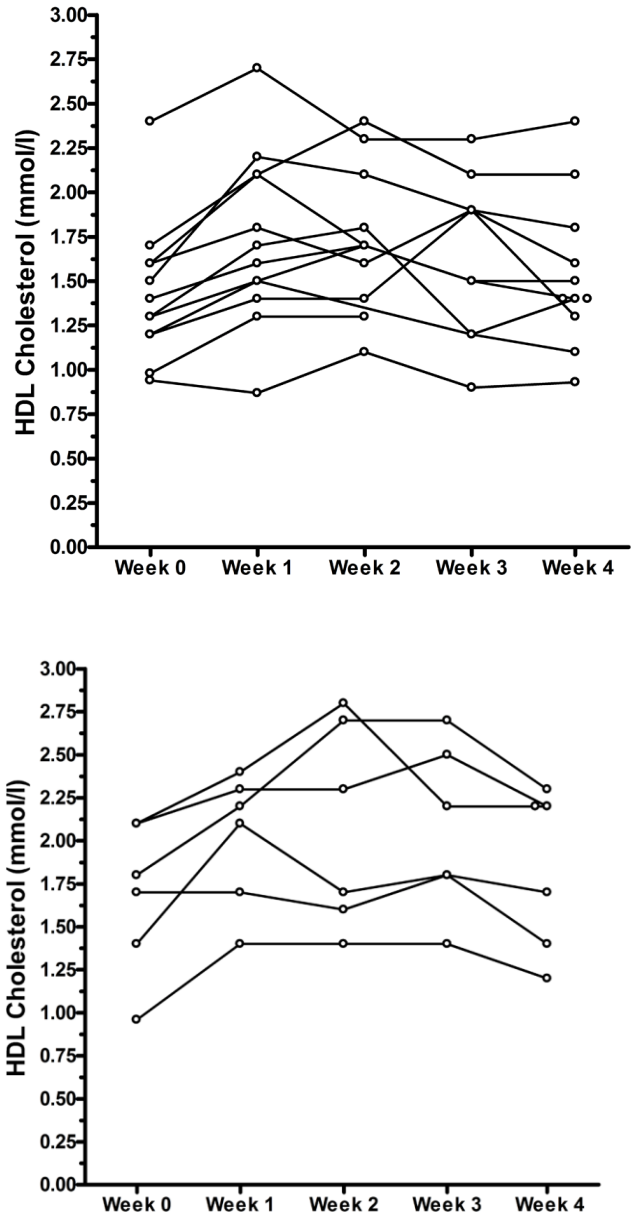

Figure 1. HDL cholesterol levels at baseline and during the study in men (a, n= 12) and women (b, n= 6) of the intervention group.

Due to the pronounced changes in insulin levels and liver enzymes (8), long term follow up was conducted of several fasting laboratory parameters in the intervention group and this showed restitution of baseline HDL cholesterol (1.4 ± 0.48 mmol/l, p= 0.3 compared with baseline) while LDL (2.5 ± 0.16 mmol/l, p= 0.02 compared with baseline), and total cholesterol (4.3 ± 0.72 mmol/l, p= 0.04 compared with baseline) were higher than at the study baseline. Insulin levels (33.8 ± 16 pmol/l, p= 0.3 compared with baseline) and triglycerides (0.81 ± 0.21 mmol/l, p= 0.2 compared with baseline) at long term follow up, on the other hand, were very similar as at the study start. The long term follow up of blood samples was performed after half a year in 13 subjects, after one year in four subjects and after 21 months in one participant, due a long trip abroad. Body weight at long term follow up at 6 or 12 months (as available) was 1.7 kg higher than at baseline (mean of 69.3 ± 8.7 kg, p= 0.008 compared with baseline).

Table 2 displays food intake and the relative composition of macronutrients before and during the study in the intervention group. The intake of saturated fat during three days at the end of week 1 was positively related to the increase in HDL cholesterol measured in the second week while a similar trend was seen for intake during week 3 (week 1: r= 0.53, p= 0.028, week 3: r= 0.46, p= 0.062). Intake of total fat during the same time periods tended to relate to the increase in HDL cholesterol levels (week 1: r= 0.46, p= 0.065, week 3: r= 0.42, p= 0.09). Intake of mono- or poly-unsaturated fatty acids at week 1 or 3 did not relate to the increase in HDL cholesterol at week 2 (all $p > 0.14$). The corresponding increase in apoA1 levels in the second week was statistically unrelated to the fat intake, and there was also no statistically significant correlation between the increase in HDL cholesterol and ApoA1 in the second week in relation to baseline (r= 0.21, p= 0.4).

Table 2. Food composition before and during the end of the first week of hyper-alimentation in the intervention group. Figures are means (SD). All changes compared to baseline were statistically significant except those of the energy % from carbohydrates.

Time period	Energy from fat, carbo-hydrates and protein (kcal/day)	Energy from fat (kcal/ day)	Energy from fat (%)	Energy from carbo-hydrates (kcal/day)	Energy from carbo-hydrates. (%)	Energy from protein (kcal/day)	Energy from protein (%)
Baseline	2273 (558)	817 (240)	36 (5.7)	1099 (297)	48 (5.4)	357 (84)	16 (1.8)
End of Week 1	5753 (1495)	2457 (728)	43 (6.8)	2575 (743)	45 (7.2)	721 (249)	12 (2.2)

DISCUSSION

Our study was primarily designed to call forth presumed dyslipidemia by the fast food based hyper-alimentation that was combined with implementing a sedentary behavior. To our surprise, however, and despite increased body weight and levels of fasting insulin, the intervention caused a pronounced transient increase in the levels of HDL cholesterol while LDL and triglyceride levels underwent comparatively smaller changes during the intervention. Indeed, the increase in HDL cholesterol surpassed that of treatment with HMG CoA reductase inhibitors, so called statins, that are widely used and well proven for treatment of cardiovascular risk linked with dyslipidemia and was of similar magnitude as that of other drugs used for treatment of dyslipidemia (10). The effect of the intervention to increase HDL cholesterol was only temporary, however. In the long term follow up HDL cholesterol had returned to baseline levels while both LDL and total cholesterol had increased. Thus LDL cholesterol and also total cholesterol displayed a smaller but more stable increase than the transient elevation of HDL cholesterol in the intervention group. The deterioration in LDL cholesterol and total cholesterol in the long term follow up could potentially be a direct consequence of the intervention.

However, we did not analyze levels of lipids in the control subjects at this time point since this was not part of the original study design, and we can thus not exclude the possibility that the deterioration could reflect natural increases in subjects of this rather youthful age group, or that baseline lipids of the intervention group were not quite representative of their usual levels due to adaption of life style in anticipation of the forthcoming weight gain. However, the levels of lipids were indeed similar in both groups at baseline.

Although it might at a first glance seem counterintuitive that HDL cholesterol increased during this short term study, this finding is actually a mirror image of the lowering of HDL cholesterol levels found during ongoing weight loss in several earlier trials (11-15). Thus, it is possible that our findings of increased HDL cholesterol, and ApoA1 levels, were consequences of increased food intake and the continuing weight gain. The increase in HDL cholesterol was positively related to the intake of saturated fatty acids in the first part of the study. This is in line with pharmacological effects of saturated fatty acids in human primary fat cells that have earlier been shown to induce PPAR gamma receptor activity (3), which leads to increases in HDL cholesterol, an effect that is clinically apparent when using strong synthetic PPAR gamma activators such as rosiglitazone (16) and pioglitazone (5). This is of particular interest since low PPAR gamma activity has been demonstrated in intra abdominal (omental) fat cells from humans (6), the fat tissue amount which is most closely linked with presence of components of the metabolic syndrome (17,18). Also, Shai et al. have earlier shown that weight reduction based on relative high fat intake compared with low fat and high carbohydrates elevated levels of HDL cholesterol more efficiently at long-term follow up after two years (19).

The fact that HDL cholesterol was increased during ongoing weight gain also raises the possibility that this was associated with an induction of liver enzymatic activity *per se*. Indeed, a body of indirect evidence is available to support this. Advanced methods in molecular biology and genomics have identified diverse biological and clinical roles for isoenzymes of P450, once believed to be solely involved in the hepatic detoxification system (20). However, P450s also respond to elevated cholesterol by activating mechanisms which efflux cellular cholesterol, raise plasma HDL cholesterol and suppress cholesterol synthesis, causing a reduction of LDL cholesterol in plasma. Studies already in the 1970s and 1980s linked liver microsomal P450-induction with elevated levels of plasma apoA1 and HDL cholesterol (20). This was followed by the discovery that plasma levels of LDL cholesterol decreased with increasing P450 activity in the liver (21,22). Indeed, subjects undergoing therapy with drugs such as phenobarbital, primidone or phenytoin, alone or in combination, have been shown to exhibit P450-induction in the liver as well as a concurrent and parallel elevation of apoA1 and HDL cholesterol levels in plasma, thus in effect implying an upregulation of apoA1 and HDL cholesterol synthesis (23).

The liver X receptor (LXR) is a key mediator in hepatic lipid regulation and by heterodimerizing with the retinoid X receptor (RXR) (24-26) LXR is able to activate the sterol responsive element binding protein 1c (SREBP-1c), and the carbohydrate responsive element binding protein (ChREBP), which have a central role in the promotion of endogenous lipid synthesis by transcriptional induction of lipogenic genes, such as fatty acid synthase and acetyl CoA carboxylase (24-26). Subjects in our intervention group exhibited both features of reduced insulin sensitivity and increased hepatic triglyceride content (8) and therefore induction of LXR is likely to have occurred. However, there is also experimental support that LXR is a cholesterol sensor

that mediates the expression of multiple genes involved in the regulation of cellular cholesterol homeostasis (27). The LXR-induced transcription of ATP-binding cassette (ABC) transporters, such as ABCA1, G1, G4, G5 and G8, participate in intracellular cholesterol transport. It has also earlier been demonstrated that an atherogenic diet upregulates hepatic P450-enzymes, hydroxycholesterols (28) and ABCA1, again supporting the idea of a major role for the liver in the dietary modulation of HDL cholesterol levels (29). Indeed, the hyper-alimentation in our study was associated with an increase in HDL cholesterol levels in plasma despite reduced insulin sensitivity. The underlying mechanisms probably involve hepatic gene activation and enzymatic induction, which may act as an initial protection from the deleterious vascular and metabolic effects of insulin resistance.

The lack of correlation between increase in HDL cholesterol and ApoA1 levels at the second week was somewhat puzzling. However, this could have occurred by chance in this rather small study or it might be a physiological reflection of different time frames for changes in apolipoprotein levels and lipoprotein composition under these rather extreme changes in lifestyle. Despite the increase in weight and insulin levels during the study, we did not find any changes in oxidized LDL concentrations, which suggests that short-time induction of insulin resistance does not affect plasma concentration of oxidized LDL in healthy subjects. Interestingly, HDL cholesterol has been proposed to harbour antioxidant properties (30) and the increase in HDL cholesterol could thus potentially have reduced oxidization of LDL cholesterol during this short term trial. In a study by Shige et al. weight loss in obese patients with type 2 diabetes led to decreased total HDL cholesterol but a changed distribution of HDL subclasses with increased alpha1 + alpha2/pre-beta ratio, ie, the ratio of large HDL cholesterol "storage" HDL to the HDL

"shuttle" fraction (31). Unfortunately we did not analyze HDL subclasses in our study since we had not anticipated such pronounced changes of HDL cholesterol levels by the intervention.

Almost all participants showed an increase in HDL cholesterol after just one week of hyper-alimentation. This is an important finding in the clinical setting when using HDL cholesterol as a measure of CVD risk. If the blood sample is taken in a period with sudden increase in caloric intake in combination with less physical exercise, the CVD risk could be underestimated, due to the temporary increase in HDL cholesterol. Also, when faced with a surprisingly high HDL cholesterol level compared with regular levels in an individual patient, it might shed light on this finding if the patient is asked whether he or she had eaten more high caloric food, especially if this was high in saturated fat, during a period ahead of the time that the sample was taken.

The first measurement of HDL cholesterol levels in our study was performed one week after study start. Therefore, we can not rule out that the HDL cholesterol levels might increase even earlier in hyper-alimentation. It would be clinically most useful to learn more about the actual time frames for the increase in HDL cholesterol during hyper-alimentation and this could be the aim of future clinical trials.

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Professor Peter Strålfors Department of Cell Biology and professor Toste Länne, Department of Medical and Health Sciences, Linkoping University.

Disclosure

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