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Serum Lipids of castrated rats given hormonal replacement and fed diets with added soybean oil or palm oil

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The effects of castration with/ without testosterone replacement in male rats, and ovarectomy with oestrogen replacement in female rats, on serum lipids were studied. Simultaneous feeding with diets fortified with 20% weight/ weight (w/ w) soybean oil (Sb) or palm oil (P0) were done to determine the influence of these oils on serum lipids in castrated and sex hormone replaced rats. Two month old male and female *Rattus norwegicus* rats were given the above treatment for 4 months, and their sera assayed for lipid profile. Castration increased HDL-cholesterol (HDLchol) and total cholesterol (Tchol) concentrations. Testosterone or oestrogen replacement in male and female rats respectively increased HDLchol and decreased LDL-cholesterol (LDLchol) concentrations. Testosterone replacement also decreased Tchol concentration back to noncastrated levels, and reduced serum triglycerides (TG) to lower than non-castrated levels. Addition of Sb or P0 to the diet increased the LDLchol in the testosterone or oestrogen replaced male and female rats, but there was no difference between the two groups. P0 raised serum TG of the testosterone replaced group compared to control and Sb groups. In conclusion, testosterone and oestrogen were found to have favourable effects on serum lipids. Sb and P0 did not differ in their effects on lipoprotein cholesterol and Tchol, but P0 raised serum TG as compared to Sb.

Introduction

Oestrogen and testosterone have been found to influence serum lipids. Oestrogen was found to increase HDL-cholesterol (HDLchol) in humans[1-3], and monkeys[4], and to decrease LDL-cholesterol (LDLchol) in humans[3]. Total cholesterol (Tchol) decreased with oestrogen administration[3,4], but no change was seen in serum triglyceride (TG) concentrations[3].

Sorva et al[5] found increased activity of hepatic lipase which was negatively correlated with serum HDLchol concentrations in pubescent boys with high androgen: oestrogen ratios. However, other researchers found that men with high levels of serum testosterone also had high levels of Tchol[6] and HDLchol[6,7], and low levels of LDLchol and TG[7].

The evidence suggests that oestrogen has a beneficial effect on serum lipids, while the effect of testosterone is still inconclusive.

Dietary fat has been shown to have an important influence on serum lipids. Higher quantities of saturated fats were found to adversely affect serum lipids as compared to higher quantities of monounsaturated palm oil (PO)[8-10], even though other reports differ[11]. On comparing PO with the more polyunsaturated soybean oil (Sb), Sundram et al[12] found that adding PO to the diet of rats increased the HDLchol and Tchol concentrations as compared to adding Sb. However, Marzuki et al[13] did not detect any difference in Tchol, HDLchol and LDLchol concentrations in adolescent boys given Sb or PO, but serum TG was higher in the Sb fed subjects.

In this study we investigated the effects of castration with/ without testosterone replacement, and ovarectomy with/ without oestrogen replacement on serum lipids of male and female rats fed diets with added Sb or PO.

Materials and Methods

Animals and castration

Male and female *Rattus norwegicus* rats, weighing between 145-165g (age: approximately 2 months) were used. Orchidectomy was done via the scrotum and ovarectomy was done through laparotomy under Pentobarbitone Sodium 35mg/ kg. A group of non-castrated male rats were used as normal controls.

Hormone preparations

Testosterone propionate (Halewood Chemicals, Middlesex, England) and beta-estradiol (Sigma, St. Louis, USA) were dissolved in corn oil (Mazola, CPC/ AJI, Kuala Lumpur, Malaysia). One mg testosterone in 0.1ml oil was injected subcutaneously to the castrated male rats every morning[14]. The ovarectomised female rats were given 25μg oestrogen in 0.1ml oil subcutaneously every morning[15]. The groups not given hormonal replacement were given 0.1ml corn oil injections daily.

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Diets

The non-castrated male rats, castrated male rats, castrated male rats given testosterone, ovarectomised female rats and ovarectomised female rats given oestrogen were fed 3 types of diets respectively. They were either fed normal diet (Gold Coin, Port Klang, Malaysia, Table 1) or normal diet fortified with 20% w/ w Sb (Yee Lee Corporation, Ipoh, Malaysia) or P0 (palm olein, Lam Soon, Petaling Jaya, Malaysia). The diets and hormonal injections were started concurrently 1 week post castration. The approximate fatty acid composition of the oils were given in Table 2. There were eight rats in each group and all rats survived the duration of experiment. The treatment was carried out for 4 months upon which the rats were exsanguinated under Pentobarbitone Sodium 35 mg/ kg and the serum lipids assayed.

Table 1. Approximate composition of rat feed

Contents	Composition % w/ w
Crude protein (min)	20.0
Crude fibre (max)	5.0
Crude fat (min)	2.5
Moisture (max)	13.0
Ash (max)	7.0
Calcium	0.7-1.4
Total phosphorous	0.6-1.2
Nitrogen-free extract	51.0

(by courtesy of Gold Coin, Port Klang, Selangor, Malaysia)

Table 2.

Fatty acid	Percent of total fatty acids (%)	
	Palm olein	Soybean oil
12:0	0.2	0.1
14:0	1.0	0.1
16:0	38.2	10.5
18:0	4.0	4.0
18:1	43.2	21.5
18:2	10.8	55.5
18:3	0.2	7.8
20:0	0.4	0.4
Saturated	43.8	15.1
Monounsaturated	43.2	21.5
Polyunsaturated	11.0	63.3

(adapted from Marzuki et al[13])

Measurement of serum lipid profile

The parameters measured were T-chol, TG and HDL-chol. The analyses were done using kits (Boehringer Mannheim, Germany). All measurements were made using Hitachi 717 computerised auto analyser. LDL-chol concentration was obtained by calculation.

Analysis of data

The results obtained were analysed via analysis of variance and Student's t test. $p<0.05$ was considered significant. This study was approved by the Research and Ethical Committee, Medical Faculty, Universiti Kebangsaan Malaysia, and confirmed by the University's Central Research Committee.

Results

Effects of castration and sex-hormone replacement on serum lipids

Castration in male rats was found to increase HDLchol concentrations, but did not change LDLchol concentrations. The HDLchol:LDLchol ratio was increased with castration. The Tchol concentration increased on castration, but no change was observed in the TG concentration (Table 3). Replacement with testosterone did not change the HDLchol concentration, but decreased LDLchol concentration; therefore increasing the HDLchol:LDLchol ratio. With testosterone replacement, Tchol concentrations decreased to non-castrated levels. TG concentrations also decreased to lower than the non-castrated and castrated levels (Table 3).

Table 3. Serum lipids of non-castrated, castrated and castrated + testosterone male rats given normal diet

Lipid profile (mmol/l)	non-castrated	castrated	castrated + testosterone
HDL-chol	0.51±0.06	*1.04±0.22	*0.92±0.12
LDL-chol	0.67±0.12	0.69±0.21	#*0.31±0.11
HDL-chol / LDL-chol	0.76±0.13	*1.59±0.41	#*3.20±0.93
T-chol	1.3±0.2	*1.9±0.4	#1.3±0.2
TG	0.70±0.22	0.77±0.21	#*0.48±0.12

Values with marker * are different from non-castrated values at $p<0.05$
Values with marker # are different from castrated values at $p<0.05$
Values are mean +SD (n = 6-8)

In female rats, replacement with oestrogen increased HDLchol concentrations, decreased LDLchol concentrations, thus increasing the HDLchol:LDLchol ratio. No change was observed in Tchol and TG concentrations (Table 4).

Table 4. Serum lipids of ovarectomised and ovarectomised + oestrogen rats given normal diet

Lipid profile (mmol/l)	ovarectomised	ovarectomised + oestrogen
HDL-chol	1.25±0.15	*1.75±0.15
LDL-chol	0.90±0.28	*0.34±0.12
HDL-chol / LDL-chol	1.51±0.51	*5.54±1.45
T-chol	2.2±0.3	2.2±0.2
TG	0.47±0.11	0.55±0.14

Values with marker * are different from ovarectomised values at $p<0.05$
Values are mean +SD (n = 6-8)

Effects of added edible oils on serum lipids of castrated and sex-hormone replaced rats.

In the castrated male rats, the PO group showed a higher HDLchol:LDLchol as compared to control group on normal diet. No significant differences were observed in the other parameters. In the testosterone replaced male rats, addition of Sb or PO in the diet increased the LDLchol concentrations and HDLchol:LDLchol ratio. Addition of Sb or PO also increased the TG concentration, and the TG concentration in the PO group was higher than the Sb group (Table 5).

There were no significant differences in serum lipids between the three diet groups of ovarectomised female rats. In the oestrogen replaced female rats, LDLchol

Table 5. Serum lipids of castrated and castrated + testosterone male rats fed normal diets and diets with added 20 % w/ w soybean oil or palm oil.

Diet	Lipid profile (mmol/ l)				
	HDL-chol	LDL-chol	HDL-chol / LDL-chol	T-chol	TG
Castrated					
Normal	1.04±0.22	0.69±0.21	^{a}1.59±0.41	1.9±0.4	0.77±0.21
Sb	0.76±0.07	0.54±0.08	2.15±0.54	1.8±0.2	0.56±0.22
PO	1.28±0.11	0.56±0.11	^{a}2.38±0.67	2.0±0.2	0.58±0.14
Castrated + testosterone					
Normal	0.92±0.12	^{bc}0.31±0.11	^{dc}3.20±0.93	1.3±0.2	^{f}0.48±0.12
Sb	0.76±0.07	^{b}0.54±0.15	^{d}1.52±0.54	1.4±0.2	^{g}0.35±0.10
PO	0.90±0.20	^{c}0.49±0.15	^{e}1.97±0.66	1.5±0.2	^{fg}0.76±0.23

Values bearing the same alphabetical superscript are significantly different at $p<0.05$. Values are in mean ± SD (n = 6-8)

Table 6. Serum lipids of ovarectomised and ovarectomised + oestrogen female rats fed normal diets and diets with added 20% w/ w soybean oil or palm oil.

Diet	Lipid profile (mmol/ l)				
	HDL-chol	LDL-chol	HDL-chol / LDL-chol	T-chol	TG
Ovarectomised					
Normal	1.25±0.15	0.90±0.28	1.51±0.51	2.2±0.3	0.47±0.11
Sb	1.23±0.28	0.84±0.16	1.52±0.48	2.2±0.3	0.45±0.07
PO	1.31±0.31	0.69±0.20	2.01±0.63	2.2±0.5	0.45±0.11
Ovarectomised + oestrogen					
Normal	1.75±0.15	^{ab}0.34±0.12	^{cd}5.54±1.45	2.2±0.2	0.55±0.14
Sb	1.60±0.21	^{a}0.59±0.10	^{c}2.78±0.67	2.3±0.2	0.48±0.07
PO	1.76±0.56	^{b}0.66±0.18	^{d}2.71±0.72	2.6±0.7	0.65±0.15

Values bearing the same alphabetical superscript are significantly different at $p<0.05$. Values are in mean ± SD (n = 6-8)

concentration and HDLchol:LDLchol ratio of the Sb and PO groups were higher than the group on the normal diet (Table 6).

Discussion

In this study, we observed that castration increased the HDLchol concentration, while testosterone replacement maintained the increased HDLchol concentration and reduced LDLchol levels, both changes being beneficial in terms of risk of developing atherosclerosis. This finding agrees with Gutai et al[7] who found that high levels of plasma testosterone correlated positively with HDLchol levels in middle-aged men. On the other hand Sorva et al[5] found an association between high levels of testosterone and low levels of HDLchol in pubescent boys whose sex steroid production was stimulated by hCG injections. Since we studied castrated rats, the effects seen here could be due to deficiency of other hormones produced by the testis besides testosterone, such as dehydroepiandrosterone and andros-tenedione. Testosterone itself could be beneficial, as seen from the HDLchol and LDLchol concentrations. Replacement with testosterone did not bring the levels of these lipoproteins back to non-castrated levels, indicating the role of other testicular products besides testosterone. High levels of androgens were found to be associated with increased activity of hepatic lipase[5,16], one of the enzymes involved in lipoprotein metabolism. Whether the changes in lipoprotein levels in our study were associated with changes in hepatic lipase and lipoprotein lipase activity can be further investigated.

Castration increased T-chol concentrations, and testosterone replacement brought it down to the non-castrated levels. This coincided with our results on serum lipoproteins, where testosterone appeared beneficial. However Nordoy et al[6] observed that high levels of testosterone was associated with high levels of T-chol in adult men. Our study also showed that testosterone replacement reduced serum TG levels, a finding which agreed with Gutai et al[7] Other studies did not find any correlation between androgens and serum TG concentrations[16,17].

We did not study serum lipids in non-ovarectomised female rats because their hormone levels fluctuate according to the menstrual cycle of each rat. In this study we found oestrogen replacement to be beneficial overall in terms of serum lipoprotein levels, while not affecting serum T-chol and TG concentrations. Similar results were observed in ovarectomised women given oestrogen replacement[3] and in users of oestrogen containing oral contraceptives[1].

Addition of 20% w/ w Sb or PO prevented the reduction seen in LDLchol concentrations of the testosterone and oestrogen replaced male and female rats. This indicates that a high fat diet has a detrimental effect on serum lipoproteins. However, there was no difference between the groups fed Sb or PO in both male and female rats. These results agree with our previous study and with Marzuki et al[13], but differ with Sundram et al[12] who observed that PO raises HDLchol compared to Sb in rats. However, PO was found to increase serum TG levels as

compared to control rats and those fed Sb in the testosterone replaced group. No significant difference was seen in the other groups studied. Other researchers did not find any significant difference in serum TG between rats fed Sb and PO diets[12], while others found that Sb raised serum TG as compared to PO[13]. Our previous study also showed that PO raised serum TG as compared to Sb.

While addition of oil to the diet was unfavourable in terms of serum lipids, greater amounts of polyunsaturated Sb did not differ from greater amounts of monounsaturated PO on their influence on serum lipoproteins and Tchol. Thus, the effect of dietary oils on serum lipids cannot be extrapolated just from the P:S ratio of each oil. The oils must be studied individually. This is because other factors may have a significant influence, such as the type of and sequence of fatty acids, as well as the position of the first double bond on the fatty acid chain. The presence of natural antioxidants also play an important role. Sb oil is rich in tocopherol, while PO is rich in tocotrienol. Tocotrienol extract from PO, and tocopherol have been shown to be favourable on serum lipids[18-22]. It is interesting to note that addition of Sb or PO attenuated the favourable decrease in LDLchol seen in the testosterone and oestrogen replaced rats as compared to the castrated and ovarectomised rats respectively.

In conclusion, both testosterone and oestrogen were found to have favourable effects on serum lipids. Fat enriched diets were unfavourable on serum lipids. Sb and P0 did not differ in their effect on serum lipoproteins and Tchol, but P0 raised serum TG levels as compared to Sb.

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Serum Lipids of Castrated Rats given Hormonal replacement and fed Diets with added Soybean oil or Palm oil.

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用激素代替並喂以豆油及棕榈油膳食大鼠的血清脂類水平

摘要

作者選用兩個月大的 Rattus Norwegicus 大鼠爲對象，先把雄鼠閹割，雌鼠切除卵巢，用激素代替，研究了對血清脂類的影響。同時喂以強化 20% 豆油或棕榈油（w/w）的膳食，四個月後再測定這些油對大鼠血清脂類水平的影響。結果發現，閹割會增加血清高密度脂蛋白膽固醇和總膽固醇濃度，分別用睾酮或雌激素代替的雄鼠與雌鼠，其高密度脂蛋白膽固醇濃度升高，而低密度脂蛋白膽固醇濃度下降。用睾酮代替的雄鼠，其總膽固醇濃度降至非閹割時水平，同時其血清甘油三酯降至比非閹割時低。加入豆油或棕榈油在膳食中會增加雌鼠和雄鼠的低密度脂蛋白膽固醇，但兩組無區別。與對照組及豆油組比較，棕榈油組會升高睾酮代替的雄鼠的血清甘油三酯水平。作者得出結論：睾酮和雌激素對血清脂類具有利影響，豆油和棕榈油對脂蛋白膽固醇和總膽固醇的影響無區別，但與豆油組比較，棕榈油會升高血清甘油三酯水平。

Abstrak

Kesan pengembirian dengan/ tanpa gantian testosteron pada tikus jantan, dan ovarektomi dengan gantian estrogen pada tikus betina, ke atas lipid serum telah dikaji. Diet yang diperkaya dengan 20% berat/ berat (b/b) minyak kacang soya (Sb) atau minyak kelapa sawit (P0) telah diberi pada masa yang sama untuk menilai kesan minyak-minyak tersebut ke atas lipid serum tikus-tikus yang dikembiri dan diberi gantian hormon seks. Tikus jantan dan betina jenis *Rattus norwegicus*, berumur dua bulan telah diberi rawatan saperti di atas selama 4 bulan, dan profil lipid serum di analisa pada akhir tempoh tersebut. Pengembirian telah meningkatkan aras kolesterol-HDL (HDLchol) dan kolesterol total (Tchol). Gantian testosteron atau estrogen pada tikus jantan dan betina masing-masing meningkatkan aras HDLchol dan mengurangkan aras kolesterol-LDL (LDLchol). Gantian testosteron juga menurunkan aras Tchol ke tahap tikus tidak dikembiri, dan menurunkan aras trigliserid (TG) serum ke tahap yang lebih rendah daripada tikus tidak dikembiri. Penambahan Sb atau P0 kepada diet meningkatkan LDLchol serum tikus-tikus jantan dan betina yang diberi gantian testosteron ataupun estrogen ketahap yang sama. Tetapi P0 telah meningkatkan TG serum kumpulan gantian testosteron ke tahap yang lebih tinggi daripada kumpulan kawalan dan Sb. Kesimpulannya, testosteron dan estrogen didapati mempunyai kesan yang menguntungkan terhadap lipid serum. Sb dan P0 tidak berbeza didalam kesan mereka terhadap aras kolesterol-lipoprotein dan Tchol, tetapi P0 meningkatkan TG serum berbanding Sb.

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